SYNTHESIS AND CHARACTERIZATION OF POLYAMIDOAMINE DENDRONS

RODERICK BORONG PERNITES
(B.Sc. Chem. Eng., Mapua Institute of Technology-Manila)

A THESIS SUBMITTED

FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

DEPARTMENT OF CHEMISTRY

NATIONAL UNIVERSITY OF SINGAPORE

2006

Acknowledgement

Despite the enormous challenges that brought the complexities in my life during my studies, still I am very much grateful with this opportunity and I don't have any regrets. Aside from the knowledge and skills that I acquired in making this experience remarkable, it brought immense discipline and introduced me to many great people that I make incredible friendships to which I owe this thesis. Allow me to thank them all:

To *Professor Sam Li* who is more than a supervisor to us, his students, I'm so much thankful for the invaluable suggestions and unwavering support that you had given me all throughout this research project and for my studies. You had been a source of great inspiration and I'm so delighted working with you.

To *Dr. Bu Jie* who is absolutely a great mentor, thank you so much for the impressive ideas, steadfast support and constant guidance throughout this work. You had taught me many important things about our research and lessons in life that I haven't learned inside the four corners of the "room". The tough questions that you had given me regarding our project had really contributed to the success of this work.

To *Mr. Zhang Huajun* who has always a piece of imposing advice, thank you so much for all your help and for the consultations that I had made with you

regarding mass spectrometry. Truly you are a valuable help to the success of this work.

To *Jeffrey Pang* who is like a kin and a young mentor, thank you so much for all the sincere help that you have shared and for all the stuff in your lab that I had borrowed. Also, the conversations with you have left me in awe of your knowledge in organic chemistry and gave me something to strive for. I really had so much fun with you learning various techniques in chemistry inside your lab. You are truly a source of inspiration in chemistry aside from being a true friend.

To the rest of the crew, Simon, Chee Wee, Chin Lee, Shou Man, Hui Fong, Elaine, Wai Siang, Mei Ching, Maricar, Norman, Shirley, Richie, Khalid, Ka Heng, Sharon Yap, Dr. Ong Teng Teng, Dr. Li Ruijang and Dr. Effendi, thank you for the laughs and support.

To my mom, dad and little sister Dhat, thank you so much for the love and support that you gave me throughout my studies. It was really tough being away from home, but your prayers had really helped me to achieve my goals and ambitions in life. Thus, I share to you this success.

To the *National University of Singapore* and *A-Star's Institute of Chemical and Engineering Sciences*, I would like to express my sincerest gratitude for the

financial $upport that you had given me for my studies and this research project including conferences expenses.

Finally to the *Almighty* who is the absolute source of everything including wisdom, and knowledge, in all honor and glory I offer this to YOU.

Table of Contents

Summary

Since the discovery of dendrimers by Vogtle[2] in the late 70's, great attention has been paid to their synthesis because of the wide spectrum of applications of the macromolecules, ranging from drug delivery systems to catalyst carriers. This is due to the high density of surface functional groups of the dendrimers that allows many synthetic and natural molecules to be attached either covalently or noncovalently to the macromolecules. The most popular class of dendrimers used in biological research is *Po*ly *AM*ido *Am*ine (PAMAM) dendrimers. They have many features common to proteins. For instance, the backbones of their chains are made up of amide linkages similar to the peptide bonds of proteins.

Following the liquid phase synthesis of PAMAM dendrimers, a new technology of synthesizing the macromolecules has evolved as a result of finding solutions into the numerous problems that are brought about by liquid-phase synthesis. Swali and co-workers started the solid-phase synthesis of PAMAM dendrons. Tsubukawa and co-workers pioneered the grafting of PAMAM dendrons into the silica gel. Bu Jie and co-workers then improved the latter method. The solid phase synthesis has the advantage of using large excess of reagents to drive the reaction to completion. The excess reagents can be easily removed by simple washing after the reaction.

Despite the progress of the silica-grafted PAMAM dendrons over the last decade, structural characterizations of the silica-supported dendrons including faulty synthesis products have not been realized previously due to the attached solid support. Therefore, chemical cleavage of PAMAM dendrons from silica gel has been done in order to perform structural analysis by using mass spectrometry, which is considered the ultimate characterization tool for these types of compounds. Furthermore, the cleavage has become a very important part of the solid-phase synthesis because of the numerous applications of the macromolecules in liquid phase.

The stepwise synthesis of PAMAM dendrons in chapter 2 has been demonstrated using alternate Michael addition and amidation reactions after immobilization of an initiator site into the silica gel. Each generation was monitored by infrared spectroscopy and Thermogravimetry. During propagation, it was suspected that side reactions occurred in both steps because theoretical amount of grafting was not achieved. It was found out that amidation step mostly contributed to the low efficiency of the synthesis. Furthermore, defects formed by side reactions cannot be distinguished from the ideal structure unless a silica gel cleavage of the macromolecule had been done followed by structural analysis through mass spectrometry.

In chapter 3, the cleavage method was investigated on a commercial compound prior to the cleavage of dendritic macromolecules on chapter 4. This silica-grafted compound has similar terminal groups as the full generation PAMAM dendron. During the cleavage of the small polymer, it was found that methanol is the most efficient solvent based on thermo-gravimetric and elemental analysis measurements. The efficiency of the cleavage is more than 80%. Therefore methanol was used as reaction solvent for cleavage of PAMAM dendrons, and the efficiency is about 90%. Finally, chapter 5 concludes the result of this research with some recommendations that are feasible for future works.

List of Tables

List of Figures

List of Schemes

.

List of Posters

1.) Poster Presentation at the 1st Postgraduate Congress; Faculty of Science, National University of Singapore ; September 21, 2005.
Title: Synthesis and Characterization of PAMAM dendrons

2.) Poster Presentation at the 1st Mathematics and Physical Science Graduate Congress 2005; Chulalongkorn University, Bangkok Thailand; December 6-7, 2005. Title: Synthesis and Characterization of Polyamidoamine dendrons

3.) Poster Presentation at the Singapore International Chemical Conference 4; Shangri-La Hotel, Singapore; December 8-9, 2005.
Title: Synthesis and Characterization of Polyamidoamine dendrons

Chapter 1: Overview of Dendrimers

1.1. What are dendrimers?

The term "**dendrimers**" is derived from two ancient Greek words: ***dendra,*** meaning tree and ***meros,*** meaning part.[1] Dendrimers portray graphically the structure of this relatively new class of macromolecules, which resembles the architecture of a tree. Dendrimers can also be referred as "**cascade polymers**" because they are synthesized through iterative steps called cascade synthesis.[2] However, the name dendrimer is the most established one.

One complete branch that makes up the structure of dendrimers is called a **dendron**[1]**.** Theoretically, an ethylenediamine-core *Po*ly *AM*ido *AM*ine (PAMAM) dendrimers are composed of four dendrons attached to the central initiator core because ethylenediamine has four reactive sites. In the case of the ammonia-core PAMAM dendrimers, there are only three dendrons attached to the core because ammonia has three reactive sites.

1.2 Properties of Dendrimers

Dendrimers are structurally well-defined highly branched three-dimensional architecture[3] with a low polydispersity[4] in comparison to traditional polymers. Dendrimers contain unique properties[5] like high degree of molecular uniformity, narrow molecular weight distribution, with specific size and shape having highly functionalized terminal groups.

Dendrimers exhibit greatly enhanced solubility in vast range of organic solvents when compared to their linear counterparts. In solution, linear chains exist as flexible coils but dendrimers form tightly packed ball. Furthermore, dendrimer solutions have appreciably lower viscosity than linear polymers.[6] As the molar mass of dendrimers increases, the intrinsic viscosity goes through a maximum at the fourth generation and then begins to decline unlike the classical polymer in which the intrinsic viscosity increases continuously with molar mass (Figure 1.1). This effect is believed to be a consequence of the globular shapes of high generation dendrimers leaving them unable to "tangle" with one another after the manner of linear polymers. [7]

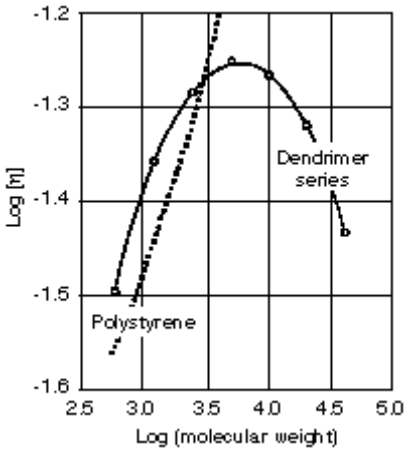


Figure 1.1 Intrinsic viscosity behaviour of polyether dendrimers and of polystyrene.[8]

The numerous chain-ends in the structure of the dendrimers play a very important role that provide the high solubility, miscibility and reactivity of the macromolecules.[6] The surface groups of the dendrimers strongly influence their solubility. For instance, dendrimers terminated in hydrophilic groups are soluble

in polar solvents while hydrophobic terminated dendrimers are soluble in non-polar solvents.

One of the most important properties of dendrimers is their ability to encapsulate guest molecules in the macromolecule interior. This was illustrated by entrapment of a small molecule *p*-nitrobenzoic acid or Rose Bengal inside the dendritic box of poly(propylene imine) dendrimer with 64 branches on the periphery (Figure 1.2). [9,10]

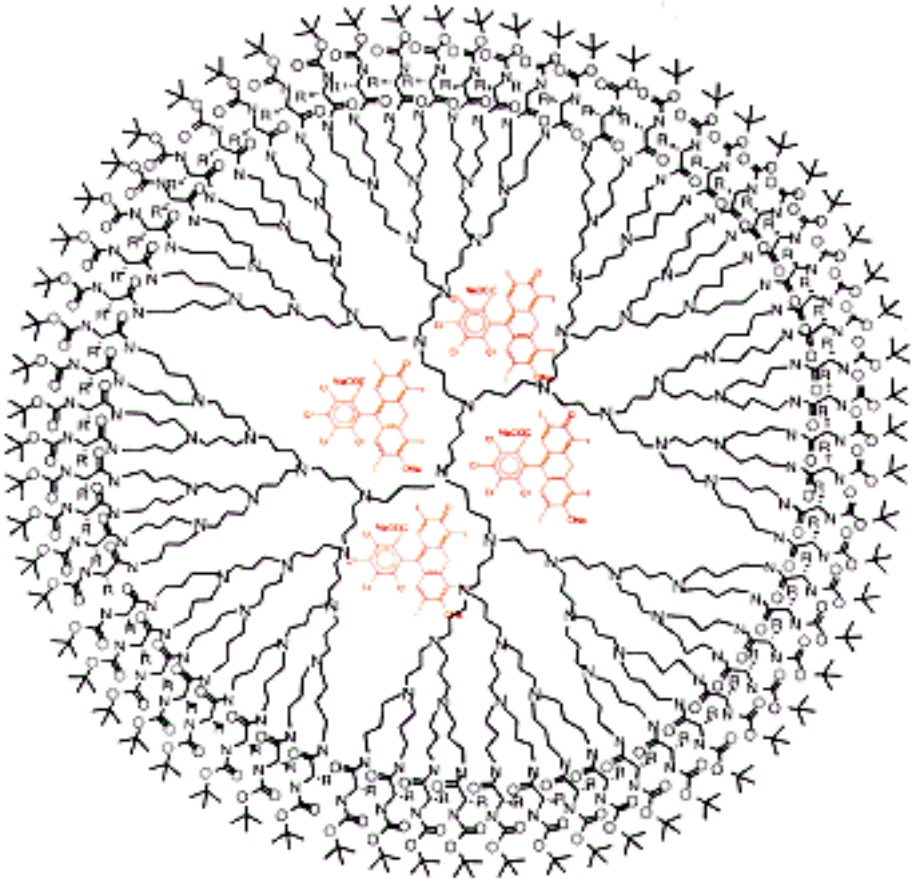


Figure 1.2 A globular dendrimer with a dense amino acid shell can hold four molecules of Rose Bengal (shown in red) and many more smaller molecules (not shown) inside the dendrimer's flexible cavities. In the structure, R = benzyl.[11]

With regards to the biological properties of dendrimers, cationic dendrimers like the amine-terminated polyamidoamine and poly(propylene imine) dendrimers are haemolytic[12] and cytotoxic[13]. Their toxicity is generation-dependent and increases with the number of surface groups.[14] However, anionic dendrimers bearing carboxylate surface are not cytotoxic over a broad concentration range.[15]

1.3 Molecular Structure of Dendrimers

The dendritic structure is characterized by layers between each focal point (or cascade) called *generation*, which is defined as the number of focal points when going from the core to the surface.[16] To illustrate, the diagram in Figure 1.3 showing the silica-supported G2.5 PAMAM dendron describes clearly the word generation.

Dendrimers of lower generations have highly asymmetric shapes and posses more open structures as compared to higher generation dendrimers.[5] They adopt a globular shape only when the macromolecule is extended outwards from the multifunctional core molecule forming more branches.[17] Dendrimers become densely packed as they extend to the periphery that forms a closed membrane-like structures.[5]

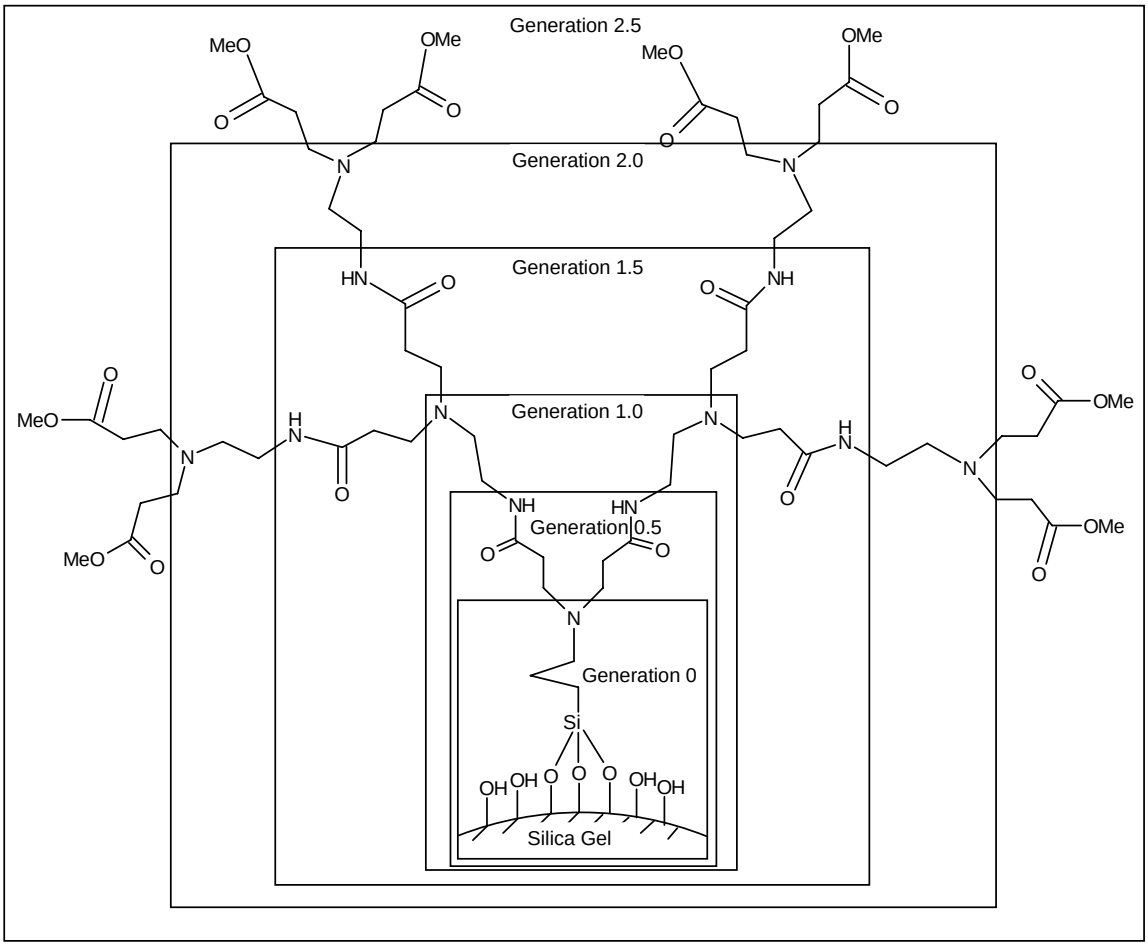


Figure 1.3 Silica-supported G2.5 PAMAM dendron showing the different generations. Half generations are enclosed in broken lines.

A typical dendrimer generation is composed of three main distinguishing structural components (Figure 1.4): (1) multifunctional central initiator core that acts as an anchor from which dendritic growth can occur, (2) internal cavities or interior layers (generations) that are composed of repeating units radially attached to the initiator core, and (3) terminal moieties (terminal functionality) from which future branching may take place.[1] These three components are

interdependent[4] with one another and reflect a unique molecular genealogy and can be tailored[5] to different sorts of applications.

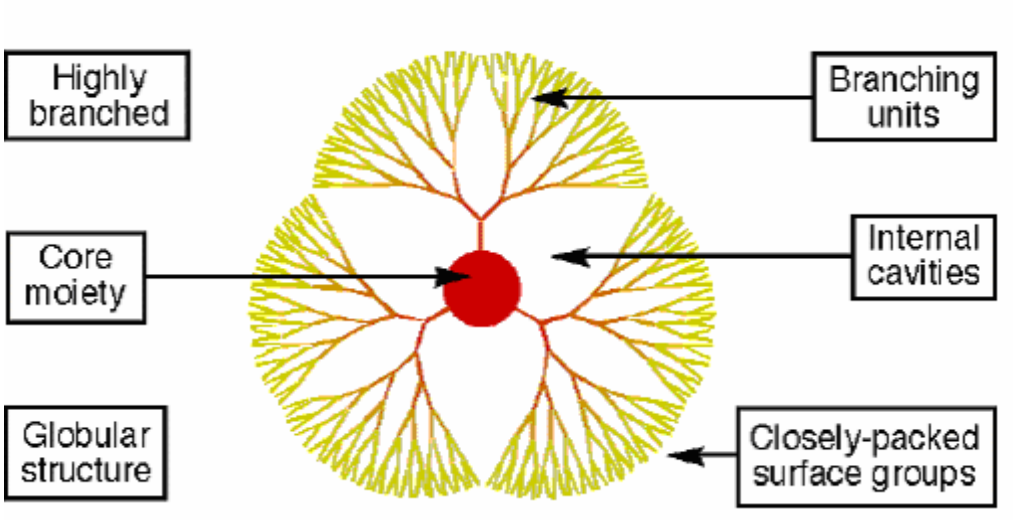


Figure 1.4 The structure of dendrimer.[18]

1.4 History and Progress of Dendrimer Research

The credit of the first dendrimer synthesis was given to Vogtle[2] and co-workers for developing the poly (propylene imine) dendrimers from acrylonitrile and aliphatic diamine in 1978. This was the first example of an iterative synthetic procedure toward well-defined branched structures, and the term “cascade synthesis” was then coined.

A few years later, Tomalia[19] and co-workers followed the synthesis of polyamidoamine dendrimer that are also known as Starburst dendrimers. The term Starburst is a trademark of the Dow Chemical Company where they made the discovery in 1979. They found out that a variety of amines could be added to methyl acrylate, and the resulting products subsequently amidated with $\propto$,ω-

diaminoalkanes would give “cascade products”.[1] This produced the Starburst dendrimers that can be started from various initiator cores like ammonia and ethylenediamine, which are two most widely used initiator cores for the synthesis of PAMAM dendrimers.

At the same time, Newkomes[20] and co-workers independently reported the synthesis of related symmetrically branched hydroxylated macromolecules that are not amendable to size control as a function of generation. They named it as arborols that originated from a Latin word “**arbor**” meaning a tree. Tomalia’s PAMAM dendrimer and Newkome’s arborol were the first dendritic structures that have been thoroughly investigated and received widespread attention in the scientific community. Both of these dendrimers were synthesized divergently from an initiator site.

The group of Frechet.[21,22] started the convergent method of dendrimer synthesis in 1990. They synthesized the aromatic polyether dendrimers. Afterwards, the group of Moore did the convergent synthesis of phenyl acetylene dendrimers.[23-26]

Kallos[27] and co-worker successfully determined the molecular weight of G4 PAMAM dendrimer in 1993 using electrospray ionization mass spectrometry (ESI-MS). The mass spectra of multiply charged ions were deconvoluted to provide molecular weight information of many components in a non-separated

mixture. Several years after, Smith[28] and co-worker studied the entire series of Starburst PAMAM dendrimers consisting of generations 1 through 10 using an extended m/z range quadrupole mass spectrometer. Furthermore, Willett[29] and co-worker had employed size exclusion chromatography with universal calibration to determine molecular weight averages, distributions, intrinsic viscosities, and structural parameters of Starburst dendrimers, dextrans and the starch degradation polysaccharides known as maltodextrins. In 2002, Peterson[30] and co-worker had used the capillary zone electrophoresis to characterize the homogeneity of individual generations of PAMAM dendrimer. A year after, they reported the use of MALDI-TOF MS in the linear and reflection mode to characterize the macromolecules including the by products, and the use of DHB/fucose as matrix had been found to give the best resolution, causing least fragmentation of the sample.[31] Currently, McLuckey[32] and co-worker had used tandem mass spectrometry to study the half-generation PAMAM dendrimer.

In 1997, Moore[33] and co-worker synthesized phenylacetylene dendrimers terminated with tert-butyl ester in a convergent approach. As compared to flexible dendrimer systems that have been shown to change size in different physical environments like that of Newkomes' polyamide dendrimer, phenylacetylene dendrimer is less susceptible to collapse because it imparts a certain stiffness or shape-persistence to the structure. In 2002, Advincula[34] and co-worker reported the design and convergent synthesis of Thiophene dendrons and dendrimers where metal-mediated coupling reactions were used in the synthesis. Following

their synthesis work, they conducted investigations on the structural characterization, size information, optical properties, and supramolecular assembly on different solid substrates of these materials.[35] In 2003, Twyman[36] and co-worker demonstrated that an aromatic AB_2 bis-amino acid monomer could be polymerized in the bulk to give a hyperbranched polymer, which is a PAMAM dendrimer equivalent. In 2004, Shabat[37] and co-worker reported the design and synthesis of new dendritic molecules with a multi-enzymatic triggering mechanism that initiates their biodegradation through self–immolative chain fragmentation to release a reporter group from the focal point. These are some of the most recent developments in the synthesis and characterization of dendrimers.

Considerable progress has been made in the area of dendrimer solid-phase synthesis over last 5 years. This type of synthesis had been discovered lately; as a result, not many works were published as compared to the liquid phase synthesis. The synthesis of PAMAM dendrimer on the solid phase starting from TentaGel™ resin was described by Swali[38] and co- workers in 1997. An acid-labile linker attached to a polyamine scaffold was used to allow cleavage of the dendrimer from the resin. On the following year, Tsubukawa[39] and co-worker grafted the PAMAM dendrons on the surface of a porous silica gel. In 2004, Bu Jie[40] and co-worker reported a method to improve the silica gel grafting of PAMAM dendrons by immobilizing first an internal standard compound that will act as a spacer in the solid support.

In the past decades, researchers had begun the exploration of dendrimers in various applications such as in biological fields. Previously, most efforts were focused on the synthesis and their chemical and physical properties. In recent years, dendrimers have shown promise in fields ranging from gene delivery to magnetic resonance imaging to the developments of vaccines, antivirals, antibacterials and anticancer therapeutic.[41-43]

For instance, Haensler and Szoka[44] carried out detailed studies on PAMAM dendrimers to generate gene-delivery vehicles in 1993. The dendritic molecules were partially functionalized with GALAcys, the cysteine-containing analogue of the amphiphilic peptide GALA, whose function was to destabilize the lipid membrane after endocytosis, thus preventing lysosomal degradation. In 1999, Twynman[45] and co-worker experimented on the release of drug from arborol-type dendrimers by calorimetric experiments, while Frechet[46] and co-workers had developed novel dendritic unimolecular micelles as drug delivery vehicles based on a core of hydrophobic Frechet-type dendrimer. Most recently, Tripathi[47] and co-worker studied the use of surface modified PAMAM dendrimers for the delivery of 5FU. Barth[48] and co-workers designed and investigated the use of boron PAMAM dendrimers as macromolecular carriers with high level of boronated MoAb required to sustain a lethal reaction at cellular level. Since dendrimers have shown promising results in a wide spectrum of potential applications, it can be expected that scientist from different interdisciplinary fields

will continuously devote intense efforts into the studies of dendrimers. The many applications of dendrimers are listed in section 1.7 of this chapter.

1.5 Method of Dendrimer Synthesis

1.5.1 Divergent and Convergent Growth

There are two conceptually different approach to synthesize dendrimers: (1) the divergent growth[49] and (2) convergent growth[50] (Figure 1.5). A repetition of reaction steps exists in both approaches that further the dendrimer growth creating a new generation, and two methodologies have their own characteristics.

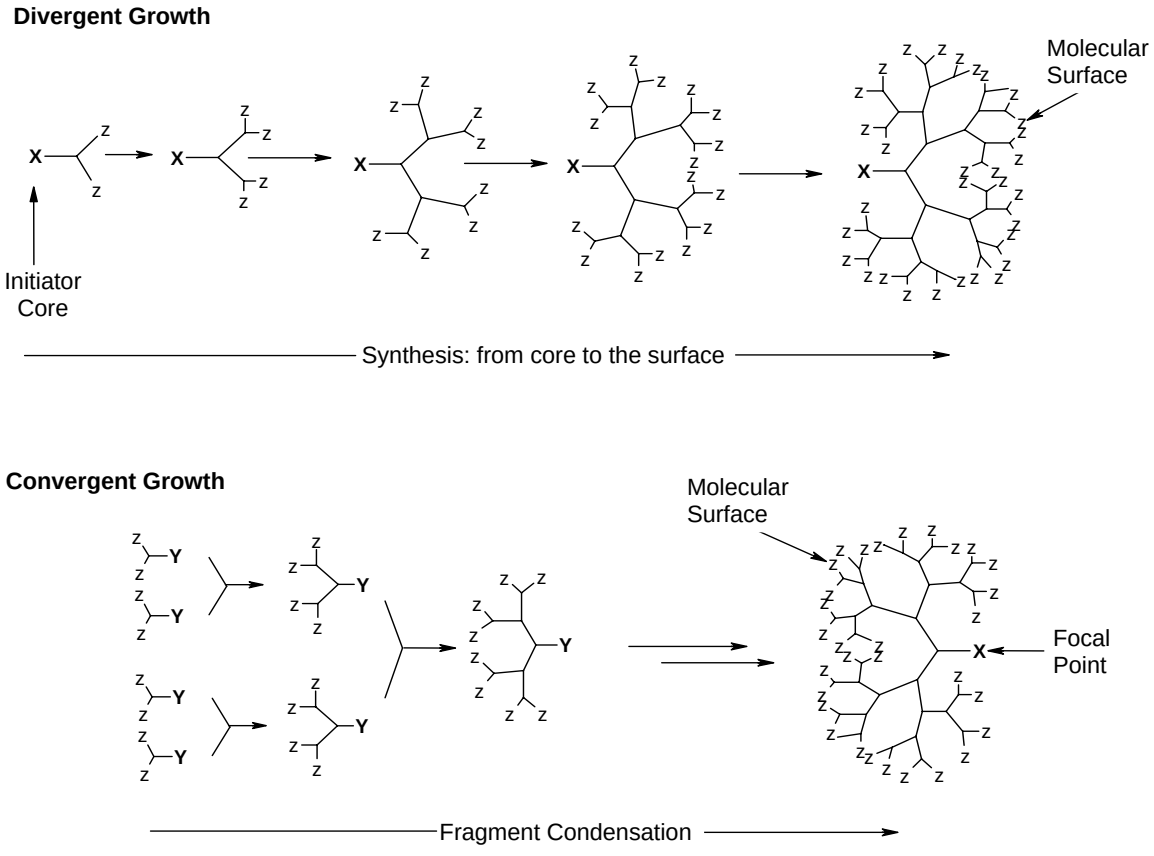


Figure 1.5 Divergent and convergent methods of dendrimer synthesis.

Divergent growth, first reported by Vogtle[2], is described by extending out the dendrimers from the initiator core via iterative reactions. As a result of this growth, each subsequent reaction is characterized by the generation of an exponentially increasing number of functional groups on the periphery.[51] The purification of dendrimers for every new generation that is divergently synthesized is difficult since numerous reactions have to be conducted on a single molecule; thus, the presence of defects cannot be avoided. In order to ensure the integrity of the final product, every reaction has to be very selective.[3] Defects are also observed in the synthesis of polypeptides or polynucleotides (Merrifield synthesis)[52] that are divergently grown on solid support. Furthermore, the propagation of PAMAM dendrons on the surface of silica gel also experienced the formation of defects during its synthesis.[40]

In the *convergent growth*, the fragments of the dendrimers are condensed together[10] followed by their subsequent addition to the core. In this approach, a constant and low number of reaction sites are warranted in every reaction step throughout the synthesis. As a consequence, only small side products can be formed in each reaction.[3] Furthermore, only relatively small dendrimers can be synthesized using this approach unlike the convergent approach in which large dendrimers are synthesized. However, purification and synthesis are easier and often more reliable than with the divergent approach.[50]

These two methods both suffer from major problems when it comes to practical synthesis.[53] As result purification becomes tedious and time-consuming because of the numerous defects formed as products of side reactions during the synthesis.

1.5.2 Liquid Phase and Solid Phase Synthesis

Dendritic macromolecules are synthesized either in liquid phase (also known as solution technology) or in solid phase. In the liquid phase, the dendrimer is synthesized directly starting from a central initiator core in a homogenous reaction. This method requires excess reagents to drive the reaction to completion, which makes subsequent purification difficult. As a result, the synthesis cost of the macromolecule increases. This contributes to the cost of the synthesis making the price of the compound expensive.

In the case of the solid-phase synthesis, a solid support is needed to propagate the dendritic macromolecule. The solid support like silica gel is functionalized with a reactive site that will serve as an anchor for the binding of the dendrimer branches called dendrons. The solid phase synthesis offers the following advantages: (1) large excess of reagents[40] can be used to drive the reaction to completion without problems associated with purification, which is a matter of extensive washing only; (2) the use of differentially protected starter units will allow an avenue into the synthesis of unsymmetrical dendrimers under very clearly defined reaction conditions and allow the synthesized dendrimer to

be specifically functionalized to other molecules of choice.[53] Furthermore, dendrimers using solid-phase technology are expected to be much more homogenous[53] than those prepared by using the conventional liquid phase technology.

The last decade has seen the rapid development[51] of the solid-phase methodology. In 1997, Swali[53] and co-workers developed the synthesis of PAMAM dendrons starting from a resin bead as a solid support. A year after, Tsubukawa[39] and his group pioneered the propagation of PAMAM dendrons on mesoporous silica gel. Lately, Bu Jie[40] and co-workers remarkably improved the silica gel propagation of PAMAM dendrons by introducing first an inert compound in the surface of the silica gel that will act as a spacer to reduce the initial density of amino groups.

1.6 Structural Defects in the Synthesis of PAMAM Dendrimer

The synthesis of PAMAM dendrimer is made up of two steps: (1) thorough Michael addition of a suitable amine initiator core with methyl acrylate and (2) exhaustive amidation of the resulting ester moieties with large excess of ethylenediamine.[19] Inevitably, there are at least three main types of primary side-reactions that cause the formation of defects in the synthesis of PAMAM dendrimer: (1) *incomplete Michael addition*[40] that produces the appearance of unsymmetrical dendritic structures or a missing arm in the structure, (2) *intramolecular cyclization*[30] that causes the formation of cross-links or cyclic

products during the synthesis of a full generation, (3) *retro-Michael addition*[19] that causes fragmentation. Furthermore, there is also the possibility of the formation of dimers (or oligomers) during the liquid phase synthesis of PAMAM dendrimer. Because of these side reactions, analysis of dendritic mixtures is complicated and they are expected to be mixtures of various components.

1.7 Applications of Dendrimers in General

The unparalleled unique characteristics of dendrimers make them suitable for high technology uses in many areas like life science, nano devices, materials, interfacial and supramolecular sciences. Further, dendrimers are now available commercially in a wide range of sizes (i.e., generations) that makes chemists in all fields interested. To simplify the presentation in this write-up, the applications of dendrimers are categorized into two major fields, namely biomedical and industrial.

1.7.1 Biomedical Field Applications

A.) **In vitro diagnostic.**[5] In the US, Dale International Inc. has developed a new method in cardiac testing. In this method, the proteins present in a blood sample bind to immunoglobulins and are fixed by dendrimers to a sheet of glass. The advantage of this method significantly reduces the waiting time for blood test results to 8 minutes instead of the usual 40 minutes process. The conjugates of dendrimer and antibody improve the precision and sensitivity of the test.

B.) Contrast agents for magnetic resonance.[54-56] Dendrimers have been evaluated for preclinical studies as contrast agents for magnetic resonance. Magnetic resonance imaging (MRI)[57] is powerful tool that captures images of the body organs and blood vessels. The addition of contrast agents increases the sensitivity and specificity of the method. For instance, dendrimers containing gadolinium ions[54,55] chelated to the surface improve visualization of vascular structures in magnetic resonance angiography (MRA) of the body.[57] This is a result of outstanding signal-to-noise ratio. Such dendrimers were found to be strong contrast agents compared to conventional agents during the preliminary investigation.[56] This work by scientists at Heribert Schmitt-Willich, Germany is about to enter Phase I clinical trials.

C.) Delivery of drugs and other therapeutic agents. A variety of molecules like drugs and other therapeutic agents can be loaded both in the interior void space and on the surface of the dendrimers to control the rate of release of these agents into the body. Sialodendrimers had shown to be potent inhibitors of the haemagglutination of human erythrocytes by influenza virus. Sialodendrimers bound to haemagglutinin prevent the attachment of the virus to cells. This prevents bacterial and viral infections.[58]

Attaching α-sialinic acid moieties to the surface of the dendrimer also enhances the therapeutic effect and enables the dendrimer to achieve higher inhibitory activity against influenza infection.[59,60] Dendrimers that are water soluble enable the binding and solubilising of small acidic hydrophobic molecules with antifungal or antibacterial properties. The substrate can be released upon contact with the target site. This is called dendrimer-based drug delivery system.[45,46]

A good example of therapeutic agent attached to the dendrimers is the boron neutron capture therapy (BNCT), which is a potential approach to cancer treatment. Dendrimers with covalently attached boron atoms have been prepared and the initial tests on these compounds showed positive results. [48, 61,62]

Conventionally, a polymer drug conjugate comprises of a linear hydrophilic polymer backbone covalently bound to a potent anti tumor drug via biodegradable spacer.[63-65] Dendrimers offer the following advantages[4] over their linear counterparts such as: (1) they possess narrow polydispersity, (2) they display the possibility to tailor-make their surface chemistry, and (3) the reduced structural density in the intramolecular core is amendable to host-guest entrapment with opportunities for subsequent release of active principles which are either water insoluble or characterized by a high toxicity.

D.) Coating agents.[66] Dendrimers can be used as coating agents to protect or deliver drugs to specific sites in the body or as time-release vehicles for biologically active agents. The 5-Fluorouracil (5FU) known to have remarkable antitumor activity has high toxic side effects. To resolve this problem, 5FU is conjugated to water-soluble *PAMAM dendrimers* through acetylation. Hydrolysis of the conjugates releases free 5FU into the site in a slow manner, reducing toxicity.

E.) Gene Therapy. It refers to the concept and practice of applying gene to treat disease. Gene therapy may be defined as a method of inserting a functioning gene into the cells of a patient to correct an inborn error of metabolism or to provide a new function in a cell. There are numerous diseases that can be treated by gene therapy including genetic defects - common illnesses such as AIDS and chronic diseases like diabetes.[67]

Dendrimers can act as carriers, known as *vectors*, in gene therapy. The purpose of vectors is to transfer genes through the cell membrane into the nucleus. Recently, liposomes and genetically engineered viruses have been mainly used for this. *PAMAM dendrimers* have been selectively chosen as genetic material carrier[68,69] because their terminal amino groups can interact with the phosphate groups of the nucleic acids. With this, consistent formation of transfection complexes is ensured.

Nowadays, commercially available transfection reagent called SuperFect™ consists of activated dendrimers. Such dendrimers can carry larger amount of genetic material than viruses.

Polyamidoamine dendrimers are capable of binding plasmid DNA and mediating gene transfer into mammalian cells.[44, 70-72] Because of their low cytotoxicity, perfectly controllable size and easy modification,[15,66,73] Polyamidoamine dendrimers find attractive application this field.

1.7.2 Industrial Applications

Besides numerous biomedical applications, dendrimers can be used to improve many industrial processes. It has been a progressing field of research and at present all these industrial applications are under study.

F.) Nanoscale Catalyst.[74] High surface area and solubility combined make dendrimers useful in nanoscale catalyst. In this case, the advantages of homogenous and heterogeneous catalysts are combined. Homogeneous catalysts are effective because of good accessibility to the active sites but purifications are difficult. On the other hand, the heterogeneous catalyst can be separated easily from the reaction mixture but the kinetics of the reaction is limited by mass transport. The advantages of using dendrimers as a catalyst are the following: (1) they have multifunctional surface and all catalytic sites are always exposed towards the reaction mixture, and (2)

they can be recovered easily from the reaction mixture by precipitation[75,76] or filtration[77,78] through a membrane.

G.) Nanostructures. Another industrial application of dendrimers that gained attention is based on nanostructures, which can be used in environment friendly industrial processes. Dendrimers can encapsulate insoluble materials like metals and transport them into a solvent within their interior. Guest molecules are trapped in the interiors of a dendrimer through hydrophobic binding[79,80], hydrogen bonding[81], metal-ligand coordination[3,82,83] and physical encapsulation[9,84]. Fluorinated dendrimers soluble in supercritical CO_2 can be used to extract strongly hydrophilic compounds from water into liquid CO_2.[85] This helps develop technologies in which hazardous organic solvents are replaced by liquid CO_2.

Cascade branch *PAMAM dendrimers* are ideal candidates as hosts of metal nanoparticles because of their fairly defined composition and structure.[86-89] The branches of the dendrimers can be used as selective gates to control the access of small molecules to the encapsulated nanoparticles.

1.8 Application of the silica-supported PAMAM dendrons

The silica-supported PAMAM dendrons synthesized in this study have significant applications in the field of catalysis[90,91] and metal complexations[92]. Immobilization of catalysts on solid supports allows easy separation from the reaction mixture by simple filtration and thus greatly facilitates the handling of the catalysts. Furthermore, the silica-supported PAMAM dendrons, which combine the merits of both the dendrimer and the inorganic support, provide ideal hosts for the preparation of highly dispersed heterogeneous catalysts, in which the particle size and binding site of the nanoparticles are well controlled. [93,94]

Previously, a dendritic catalyst for olefin epoxidation was prepared by immobilization of a Mn(II) salen complex onto the periphery of the silica-supported PAMAM dendrons.[92] Also, a PAMAM dendritic catalyst anchored onto the silica gel was used in the hydroformylation[93,94] and in the addition of diethylzinc to form benzaldehyde[95].

1.9 Scope of Research

The scope of this research is to divergently synthesize PAMAM dendrons on solid phase using mesoporous ultrafine silica gel as support and characterize them using advanced analytical techniques like infrared spectroscopy, thermogravimetry, elemental analysis and mass spectrometry. The silica gel propagation of PAMAM dendrons is achieved by alternate (1) Michael addition of methyl acrylate to the amino reactive sites of the organic phase grafted onto the

silica gel and (2) amidation reactions of the resulting ester moieties with ethylenediamnine. Prior to the two iterative steps aforementioned, the silica particles were treated with 3-aminopropyltriethoxysilane to serve as coupling agent for the propagation of PAMAM dendrons. In this research work, we decided to synthesize the macromolecule up to generation 2.5.

Following the solid-phase synthesis of PAMAM dendrons, silica gel cleavage of the full generation dendrons had been done followed by mass spectrometric analysis to characterize the structure of the macromolecules. Prior to the cleavage of PAMAM dendrons, the effectiveness of the method was investigated on a smaller silica-grafted commercial compound having the same terminal group as the full generation dendrons. During the cleavage of the small polymer, several solvents were tested for the reaction namely, THF/methanol, methanol only, and water to find the most efficient solvent that can be used for the cleavage of PAMAM dendrons grafted onto the silica gel. After finding the most suitable solvent, the cleavage of the dendritic macromolecules were done and the reaction was carried at room temperature and 60 ^{0}C.

1.10 References

1. Tomalia, D.A.; Naylor, A.M.; Goddard, W.A., III. ***Angew. Chem. Int. Ed. Engl.*** 1990, 29, 138-174.
2. Buhleier, E.W.; Wehner, W.; Vogtle, F. ***Synthesis***. 1978, 155-158.
3. Bosman, A. W.; Janssen, H. M.; Meijer, E.W.; ***Chem. Rev.*** 1999, 99, 1665-1668.
4. Lorenzo, M.; Ferrone, M.; Coslanich, A.; Fuchs, S.; Fermeglia, M.; Paneni, M.S.; Pricl, S. ***Biomacromolecules.*** 2004, 5, 1371-1378.
5. Klajnert, B.; Bryszewska, M. ***Acta Biochim. Pol.*** 2001, 48, 199-208.
6. Frechet, J.M.J. ***Science.*** 1994, 263, 1710-1715.
7. Mourey, T.H.; Turner, S.R.; Rubenstein, M.; Frechet, J.M.J.; Hawker, C.J.; Wooley, K.L. ***Macromolecules.*** 1992, 25, 2401-2406.
8. Fréchet, J.M.J.; Hawker, C.J.; Wooley, K.L.; J. M. S. ***Pure Appl. Chem***. 1994, A31 (11), 1627-1645.
9. Jansen, J.F.G.A.; de Brabander van den Berg, E.M.M.; Meijer, E.W. ***Science.*** 1994, 266, 1226-1229.
10. Jansen, J.F.G.A.; Meijer, E.W. ***J. Am. Chem. Soc.*** 1995, 117, 4417-4418.
11. Meijer, et al. ***Polym. Mater. Sci. Eng.*** 1995, 73, 123.
12. http://www.vetpathology.com/anaemia.htm
13. http://www.medterms.com/script/main/art.asp?articlekey=19883
14. Roberts, J.C.; Bhalgat, M.K.; Zera, R.T. ***J. Biomed. Material Res.*** 1996, 30, 53-65.
15. Malik, N.; Wiwattanapatapee, R.; Klopsch, R.; Lorenz, K.; Frey, H.; Weener, J.W.; Meijer, E.W.; Paulus, W.; Duncan, R. ***J. Control. Release.*** 2000, 65, 133-148.
16. Boas, U.; Heegaard, P. M. H. ***Chem. Soc. Rev.*** 2004, 33, 43-63.
17. Caminati, G.; Turro, N.J.; Tomalia, D.A. ***J. Am. Chem. Soc.*** 1990, 112, 8515-8522.
18. http://www.ninger.com/dendrimer/
19. Tomalia, D.A.; Baker, H.; Dewed, J.R.; Hall, M.; Kilos, G.; Martin, S.; Rock, J.; Ryder, J.; Smith, P. ***Macromolecules.*** 1986, 19, 2466-2468.
20. Newkome, G.R.; Yao, Z.Q.; Baker, G.R.; Gupta, K. ***J. Org. Chem.*** 1985, 50, 2003-2004.
21. Hawker, C.J.; Frechet, J.M.J. ***J. Am. Chem. Soc.*** 1990, 112, 7638-7647.
22. Hawker, C.J.; Frechet, J.M.J. ***J. Chem. Soc., Chem. Commun.*** 1990, 1010-1013.
23. Moore, J.S.; Xu, Z. ***Macromolecules.*** 1991, 24, 5893-5894.
24. Xu, Z.; Moore, J.S. ***Angew. Chem. Int. Ed. Engl.*** 1993, 32, 246-248.
25. Kawaguchi, T.; Walker, K.L.; Wilkins, C.L.; Moore, ***J.S. J. Am. Chem. Soc.*** 1995, 117, 2159-2165.
26. Moore, J.S. ***Acc. Chem. Res.*** 1997, 30, 402-413.
27. Kallos, G.J.; Tomalia, D.A.; Hedstrand, D.M.; Lewis, S.; Zhou, J. ***Rapid Commun Mass Sp.*** 1991, 5, 383-386.

28. Schwartz, B.L.; Rockwood, A.L.; Smith, R.D.; Tomalia, D.A.; Spindler, R. ***Rapid Commun. Mass Sp.*** 1995, 9, 1552-1555.
29. Striegel, A.M.; Plattner, R. D.; Willett, J.L. ***Anal. Chem***. 1999, 71, 978-986.
30. Peterson, J.; Ebber, A.; Allikmaa, V.; Lopp, M. ***Proc. Estonian Acad. Sci. Chem.*** 2001, 50, 3, 156-166.
31. Peterson, J.; Allikmaa, V.; Subbi, J.; Pehk, T.; Lopp, M. ***Eur. Polym. J.*** 2003, 39, 33-42.
32. He, M.; McLuckey, S.A. ***Rapid Commun. Mass Sp.*** 2004, 960-972.
33. Moore, J.S.; Pesak, D.J.; ***Macromolecules.*** 1997, 30, 6467-6482.
34. Advincula, R.C.; Wang, S.; Wang, X.; Li, L.; ***Org. Lett.*** 2002, 4, 2067-2070.
35. Nonidez, W.; Gies, A.; Advincula, R.C.; Locklin, J.; Fan, X.; Xia, C. ***J. Am. Chem. Soc.*** 126, 8735-8743.
36. Twyman, L.J.; King, A.S.H.; Burnett, J.; Martin, I.K. ***Tetrahedron Lett.*** 2004, 45, 433-435.
37. Shabat, D.; Amir, R.J. ***Chem. Commun.*** 2004, 1614-1615.
38. Swali, V.; Wells, N.J.; Langley, G.J.; Bradley, M. ***J.Org. Chem.*** 1997, 62, 4902.
39. Tsubukawa, N.; Ichioka, H.; Satoh,H.; Hayashi, S.; Fujiki, K. ***React. Funct. Polym.*** 1998, 37, 75-82.
40. Bu, J.; Li, R.; Quah, C.W.; Carpenter, K.J. ***Macromolecules***. 2004, 37, 6687-6694.
41. Aulenta, F.; Hayes, W.; Rannard, S. ***Eur. Polym. J.*** 39, 1741-1771.
42. Stiriba, S.E.; Frey, H.; Haag, R. ***Angew. Chem. Int. Ed.*** 2002, 41, 1329-1334.
43. Patri, A.K.; Majoros, I.; Baker, J.R.Jr. ***Curr. Opin. Chem. Biol.*** 6, 466-471.
44. Haensler, J.; Szoka, F.C. Jr.; ***Bioconjug. Chem.*** 1993, 4,5, 372-379.
45. Twyman, L.J.; Beezer, A.E.; Esfand, R.; Hardy, M.J.; Mitchell, J.C. ***Tetrahedron Lett.*** 1999, 40, 1743-1746.
46. Liu, M.; Kono, K.; Frechet J.M.J. ***J. Control Rel.*** 2000,65,121.
47. Tripathi, P.K.; Khopade, A.J.; Nagaich, S.; Shrivastava, S.; Jain, S.; Jain, N.K. ***Pharmazie.*** 2002, 57, 261.
48. Barth, R.F.; Adams, D.M.; Soloway, A.H.; Adam, F.; Darby, M.V. ***Bioconjug. Chem.*** 1994, 5, 58-66.
49. Tomalia, D.A.; Baker, H.; Hall, M.; Kallos, G.; Martin, S.; Roeck, J.; Ryder, J.; Smith, ***P. Polym. J.*** 1985, 108, 849.
50. Twyman, L.J.; King, A.S.H.; Martin, I.K. ***Chem. Soc.Rev.*** 2002, 31, 69-82.
51. Lebreton, S.; Monaghan, S. Bradley, M. ***Aldrichchim. Acta.*** 2001, 34(3), 75.
52. Solomons, T.W.G. ***Organic Chemistry.*** 6th ed.; Wiley: New York, 1996; p 116.
53. Swali, V.; Wells, N.J.; Langley, J.; Bradley, M. ***J. Org. Chem.*** 1997, 62, 4902-4903.

54. Weiner, E.C.; Auteri, F.P.; Chen, J.W.; Brechbiel, M.W.; Gansow, O.A.; Schneider, D.S.; Belford, R.L.; Clarkson, R.B.; Lauterbur, P.C. ***J. Am. Chem. Soc.*** 1996, 118, 7774-7782.
55. Bryant, L.H.; Brechbiel, M.W.; Wu, C.; Bulte, J.W.M.; Herynek, V.; Frank, J.A. ***J. Magn. Reson. Imaging.*** 1999, 9, 348-352.
56. Bourne, M.W.; Margerun, L.; Hylton, N.; Campion, B.; Lai, J.J.; Derugin, N.; Higgins, C.B. ***J. Magn. Reson. Imaging.*** 1996, 6, 305-310.
57. http://www.radiologyinfo.org/content/mr_of_the_body.htm#Description
58. Sigal, G.B.; Mammen, M.; Dahmann, G.; Whitesides, G.M. ***J. Am. Chem. Soc.*** 1996, 118, 3789-3800.
59. Roy, R.; Zanini, D.; Meunier, S.J.; Romanowska, A. ***J. Chem. Soc. Chem. Commun.*** 1993, 1869-1872.
60. Zanini, D.; Roy, R. ***J. Org. Chem.*** 1998, 63, 3486-3491.
61. Liu, L.; Barth, R.F.; Adams, D.M.; Soloway, A.H.; Reisefeld, R.A.; ***J. Hematother.*** 1995, 4, 477-483.
62. Capala, J.; Barth, R.F.; Bendayam, M.; Lauzon, M.; Adams, D.M.; Soloway, A.H.; Fenstermaker, R.A.; Carlsson, J. ***Bioconjug. Chem.*** 1996, 7, 7-15.
63. Duncan, R. ***Anti-Cancer Drugs.*** 1992, 3, 175.
64. Duncan, R.; Dimitrijevic, S.; Evagororou, E.G. ***Stp. Pharma Sci.*** 1996, 6, 237.
65. Vasey, P.; Twelves, C.; Kaye, S.B.; Wilson, P.; Morrison, R.; Duncan, R.; Thompson, A.; Hilditch, T.; Murray, T.; Burtles, S.; Cassidy, J. ***Clin. Cancer Res.*** 1999, 5, 83.
66. Zhou, R.X.; Du, B.; Lu, Z.R. ***J. Control. Release.*** 1999, 57, 249-257.
67. Culver, K.W. ***Gene Ther.*** 1996, 16.
68. Bielinska, A.U.; Kukowska-Latallo, J.F.; Johnson, J.; Tomalia, D.A.; Baker, J.R. ***Nucleic Acids Res.*** 1996, 24, 2176-2182.
69. Kukowska-Latallo, J.F.; Raczka, E.; Quintana, A.; Chen, C.L.; Rymaszewski, M.; Baker, J.R. ***Hum. Gene Ther.*** 2000, 11, 1385-1395.
70. Kukowska-Latallo, J.F.; Bielinska, A.U.; Johnson, J. ***Proc. Natl. Acad. Sci. USA.*** 1996, 93,10, 4897-4902.
71. Du, B.; Zhou, R.J.; Zhou, R.X. ***Chin. Chem. Lett.*** 1999, 9, 635-638.
72. Cheng, H.; Zhou, R.; Liu, L. ***Genetica.*** 2000, 108, 1, 53-56.
73. Esfand, R.; Tomalia, D.A. ***Drug Discov. Today.*** 2001, 6, 8, 427-436.
74. Tomalia, D.A.; Dvornic, P.R. ***Nature.*** 1994, 372, 617-618.
75. Seebach, D.; Marti, R.E.; Hintermann, T. ***Helv. Chim. Acta.*** 1996, 79, 1710-1740.
76. Reetz, M.T.; Lohmer, G.; Schwickardi, R. ***Angew. Chem.*** 1997, 109, 1559-1562.
77. Brinkmann, N.; Giebel, D.; Lohmer, G.; Reetz, M.T.; Kragl, U. ***J. Catal.*** 1999, 183, 163-168.
78. Hovestad, N.J.; Eggeling, E.B.; Heidbuchel, H.J.; Jastrzebski, J.T.B.H.; Kragl, U.; Keim, W.; Vogt, D.; Koten, G. van. ***Angew. Chem.*** 1999, 111, 1763-1765.

79. Naylor, A.M.; Goddard, W.A.I.; Kiefer, G.E.; Tomalia, D.A. ***J. Am. Chem. Soc.*** 1989, 111, 2339.
80. Newkome, G.R.; Moorefield, C.N.; Baker, G.R.; Saunders, M.J.; Grossman, S.H. ***Angew. Chem. Int. Ed. Engl.*** 1991. 30, 1178.
81. Newkome, G.R.; Woosley, B.D.; He, E.; Moorefield, C.D.; Guther, R.; Baker, G.R.; Escamilla, G.H.; Merrill, J.; Luftmann, H. ***Chem. Commun.*** 1996, 2737.
82. Newkome, G.R.; He, E.; Moorefield, C.N. ***Chem. Rev.*** 1999, 99, 1689.
83. Zeng, F.; Zimmerman, C. ***Chem. Rev.*** 1997, 97, 1681.
84. Jansen, J.F.G.A.; de Brabander van den Berg, E.M.M.; Meijer, E.W. ***Adv. Mater.*** 1995, 7, 561.
85. Cooper, A.I.; Londono, J.D.; Wignall, G.; McClain, J.B.; Samulski, E.T.; Lin, J.S.; Dobrynin, A.; Rubinstein, M.; Burke, A.L.C.; Frechet, J.M.J.; DeSimone, J.M. ***Nature.*** 1997, 389, 368-371.
86. Crooks, R.M.; Zhao, M.; Sun, L.; Chechik, V.; Yeung L.K. ***Acc. Chem. Res.*** 2001, 34, 181.
87. Zhao, M.; Crooks, R.M. ***Angew. Chem.*** 1999, 111, 375.
88. Zhao, M.; Sun, L.; Crooks, R.M. ***J. Am. Chem. Soc.*** 1998, 120, 4877.
89. Henglein, A. ***J. Phys. Chem. B.*** 2000, 104, 2201.
90. Kreiter, R.; Kleij, A.W.; Gebbink, R.J.M.K.; Koten, G. ***Top. Curr. Chem.*** 2001, 217, 163.
91. Astruc, D.; Chardac, F. ***Chem. Rev.*** 2001, 101, 2991.
92. Bu, J.; Judeh, Z.M.A.; Ching, C.B.; Kawi, S. ***Catal. Lett.*** 2003, 85, 3-4, 183-187.
93. Bourque, S.C.; Maltais, F.; Xiao, W.J.; Tardif, O.; Alper, H.; Arya, P.; Manzer, L.E. ***J. Am. Chem. Soc.*** 1999, 121, 3035.
94. Bourque, S.C.; Alper, H.; Manzer, L.E.; Arya, P. ***J. Am. Chem. Soc.*** 2000, 122, 956.
95. Chung, Y.M.; Rhee, H.K. ***Chem. Commun.*** 2002, 238.

Chapter 2: Synthesis of PAMAM Dendrons in Porous Silica Gel

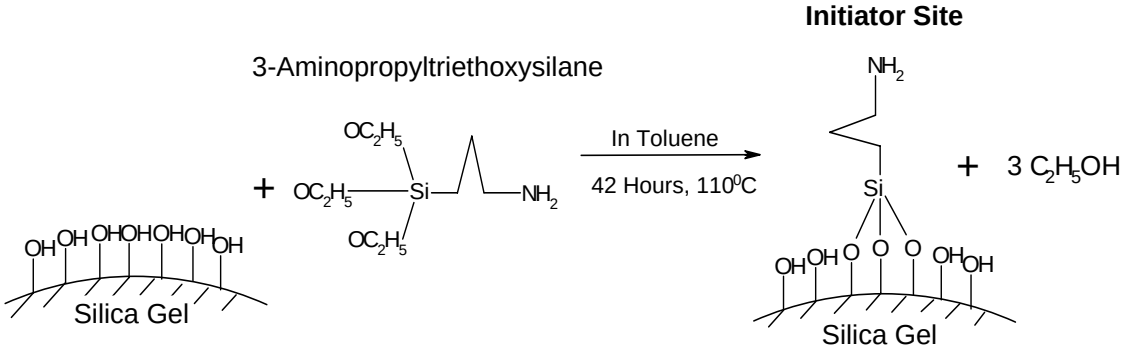
2.1. Introduction

The grafting of PAMAM dendrons was achieved by the two-step polymerization of monomers initiated by the reactive sites previously introduced onto the surface of silica gel. The two-step polymerization process includes the (1) Michael addition of amino reactive site of the organic phase grafted onto the silica gel with acryl ester and (2) Amidation of the resulting ester moieties with alkylene diamine. [1] This type of growth is called divergent because the dendron is extended out from the initiator site via iterative reactions.

2.1.1 Immobilization of Initiator Site

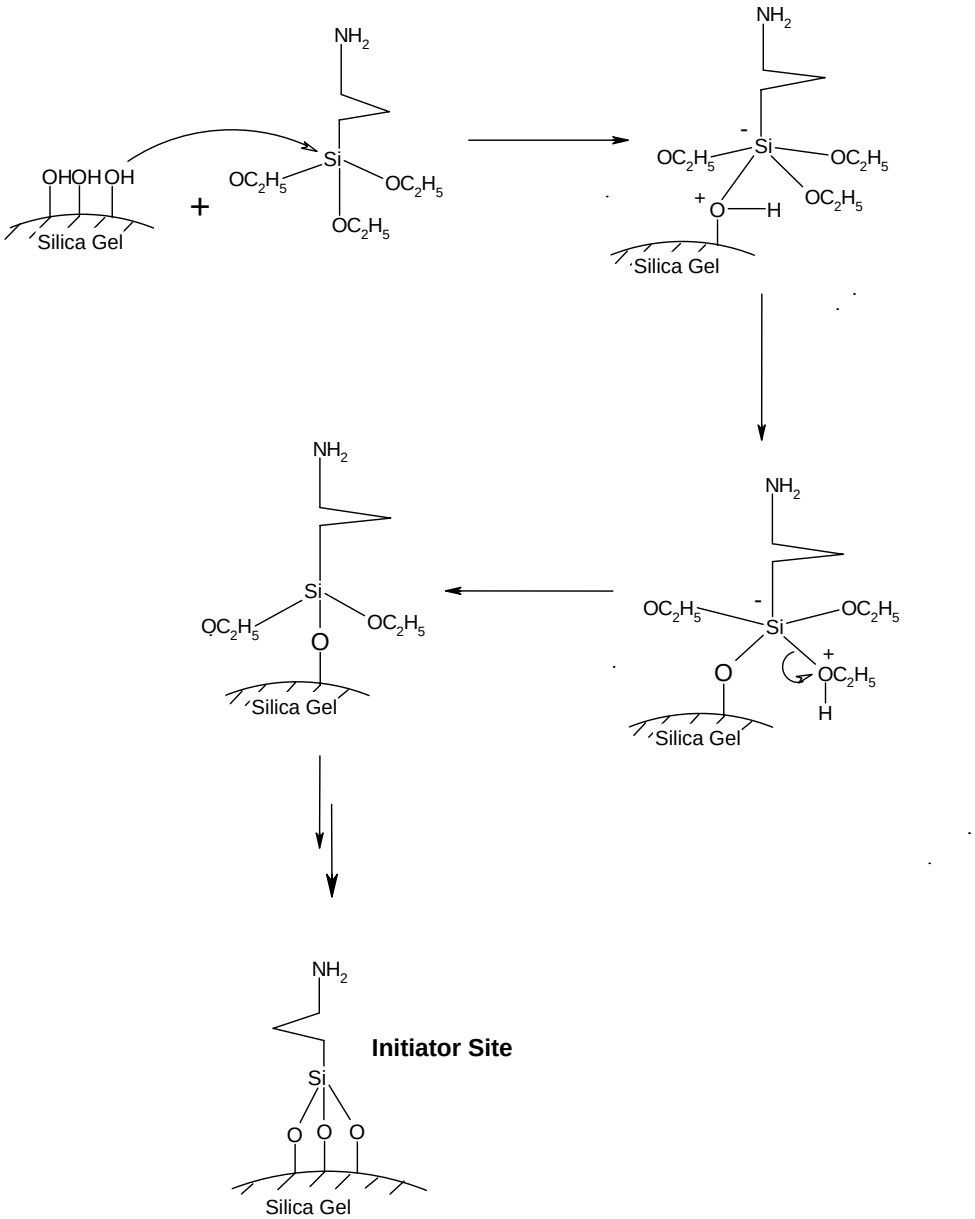
To introduce the initiator compound with amino reactive site, the mesoporous spherical silica gel was treated with 3-aminopropyltriethoxysilane (APES) that would serve as the coupling agent for the propagation of PAMAM dendron (refer to Scheme 2.1). This process is called silylation, wherein the amino functional organosilane reacts with the surface silanols of the silica gel through chemical bonding. Hence, detachment of the initiator compound is prevented because of its strong covalent attachment into the silica gel.[2] In contrast, the silane molecules that only adsorbs in the surface of the silica gel were simply removed during the experiment by washing and Soxhlet extraction with the reaction solvent.

Other studies have shown that the silylation step is usually difficult to control and non-uniform.[3] These were experienced in the experiment during the immobilization of 3-aminopropyltriethoxysilane into the silica gel. Moreover, the choice of solvent greatly affects the silylation process.[3-5] For instance, the chloro and alkoxy groups of multifunctional organosilanes undergo bulk hydrolysis and condensation in an aqueous environment to form polysilane networks before depositing onto the substrate.[3, 5] However, anhydrous silylation has the advantage of minimizing intercondensation between silane molecules in the bulk phase before being deposited into the surface of silica gel. [3,4,6] In order to have a denser and more uniform silylation coverage, the silylation step in the synthesis was done in anhydrous environment using toluene as the reaction solvent at 110 ^{0}C. Toluene is a suitable solvent for the silylation reaction because it completely dissolves 3-aminopropyltriethoxysilane, which is very useful in the vacuum filtration and Soxhlet extraction part for the removal of the excess amount. The theoretical illustration of the silylation reaction is shown in Scheme 2.1.



Scheme 2.1 Theoretical illustration of the immobilization of 3-Aminopropyltriethoxysilane into the silica gel to form the initiator compound (G0) with amino reactive site.

The oxygen in the silanol group of the silica gel attacks the silicon group of the 3-aminopropyltriethoxysilane making the oxygen becomes positively charged and the silicon a negatively charged specie. Eventually the oxygen loses the proton, and silicon loses one ethoxy group after gaining a proton. The same mechanism applies to the other two-ethoxy substituents of the silicon atom. The mechanism is depicted in Scheme 2.2.



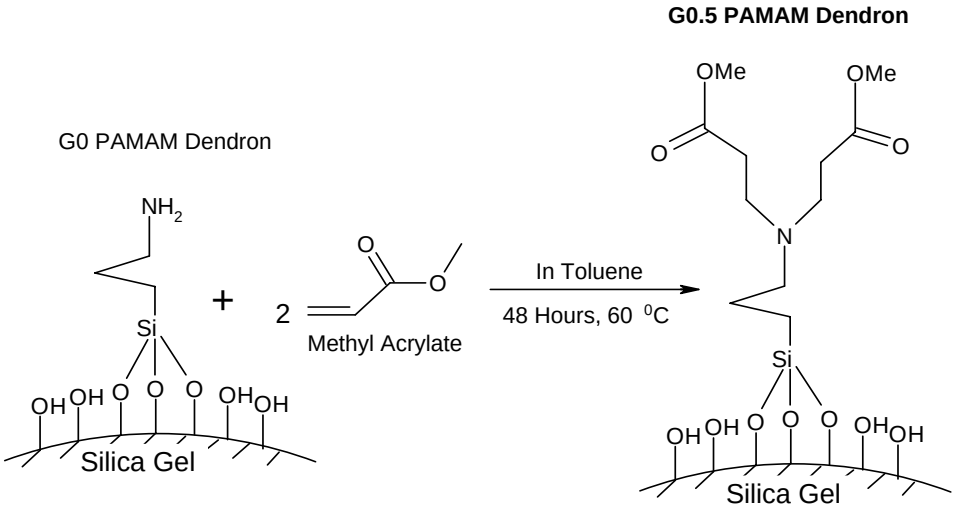
Scheme 2.2 Silylation reaction mechanism.

2.1.2. Propagation of PAMAM Dendron

A. Michael addition

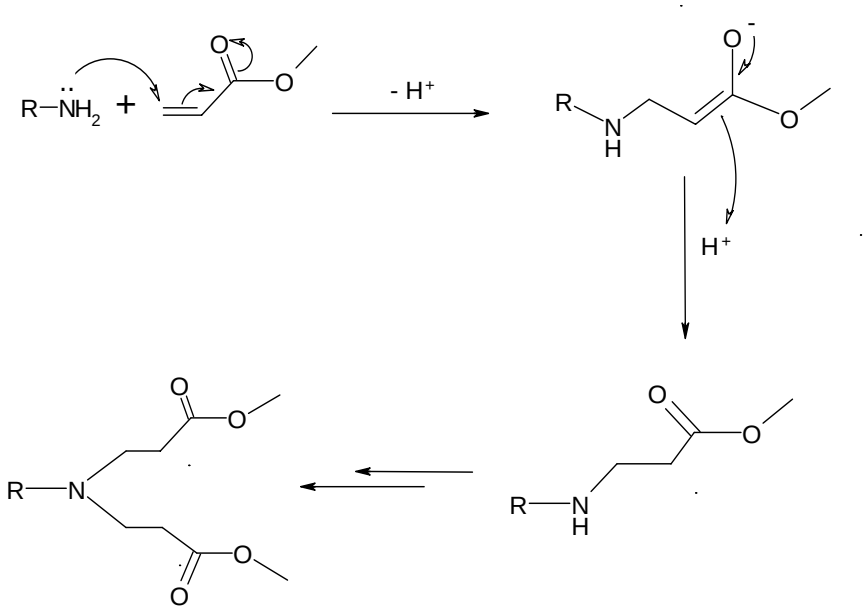
The construction of the silica-supported PAMAM dendron was carried out by the repetitive addition of a branching unit into the initiator site. First, Michael addition of methyl acrylate into the amino reactive site of the initiator compound generated the ester-terminated PAMAM dendron (G0.5). This step was done in a batch reactor at 60 ^{0}C using toluene as the reaction solvent.

When Michael addition is incomplete, structures with missing branch/branches are formed. This is due to steric hindrance exhibited by the organic phase previously grafted onto the silica gel.[1] To illustrate, the highly branched polymer grafted on silica gel blocks the addition of another monomer unit causing the new compound to have one or more missing branches. The theoretical illustration of a typical Michael addition is shown in Scheme 2.3.



Scheme 2.3 Theoretical illustration of the Michael addition of the amine terminal group of the initiator site with Methyl Acrylate to form the G0.5 ester terminated PAMAM dendron.

The amino group on the silica-grafted dendron attacks the β carbon on methyl acrylate via the Michael Addition reaction mechanism. As a result the dendron forms the half-generation, which is ester terminated. The reaction mechanism is shown in Scheme 2.4.



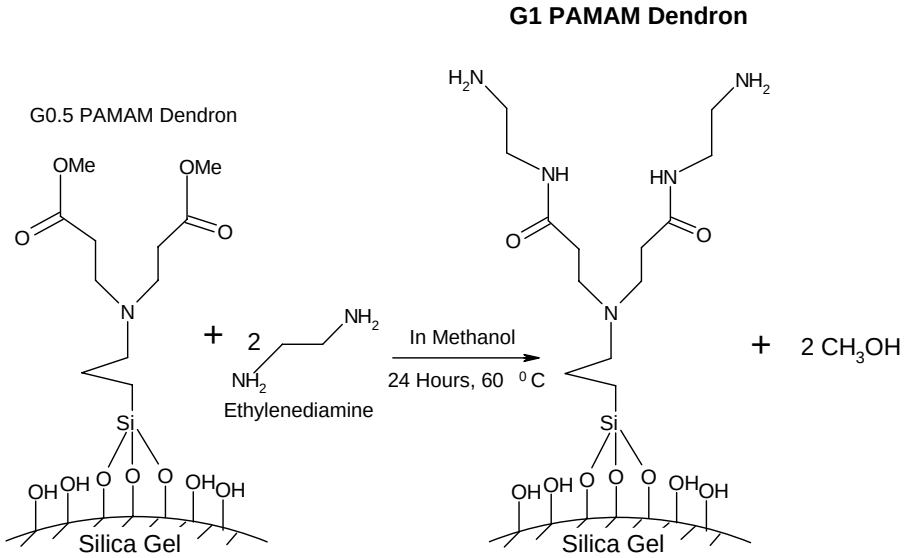
Scheme 2.4 Michael addition reaction mechanism.

B. Amidation

Amidation of the resulting ester terminal groups with large excess ethylenediamine completed the first generation (G1). This reaction was also carried out in a slurry batch reactor at 60 ^{0}C using methanol as the reaction solvent.

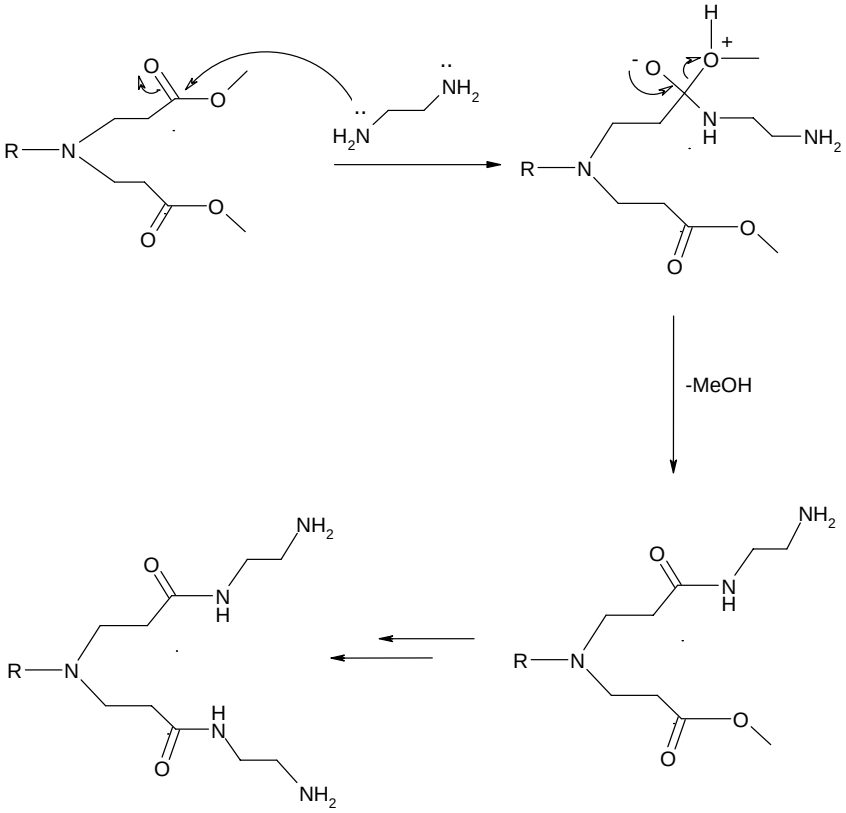
A side reaction called cross-linking competes with amidation, producing another structural defect.[7] When cross-linking happens on the same branch, wherein the two-ester terminal groups adjacent to each other are being linked by

one molecule of ethylenediamine, this is called intramolecular cyclization.[11] Cross-linking is also possible between the two-ester terminal groups of neighboring branches as shown in Figure 2.6. Hence to minimize cross-linking, a large excess of ethylenediamine was used in the experiment to drive the reaction to completion. Ethylenediamine is particularly suitable for the amidation step because its boiling point (bp 110 ^{0}C) allowed the removal of the large excesses under conditions that would not alter the dendrimer/dendron structure.[12] The theoretical illustration of a typical amidation reaction is shown in Scheme 2.5.



Scheme 2.5 Theoretical illustration of the amidation reaction of the ester terminal group of G0.5 by addition of ethylenediamine to form G1 PAMAM dendron.

One of the terminal amine groups on ethylenediamine attacks the carbonyl carbon of the ester moiety on the dendron, forming an amide bond and causing the elimination of a methanol molecule. The same mechanism happens on all other ester moieties. Finally, the dendron on the surface of the silica gel becomes amine terminated, hence completing the full generation. The reaction mechanism of the amidation is shown in Scheme 2.6.



Scheme 2.6 Amidation reaction mechanism.

An alternate repetitive Michael addition of the amino reactive site with methyl acrylate and Amidation of the ester moieties using ethylenediamine form the other generations depicted in Figure 2.1. A representative reaction of the propagation of PAMAM dendron in the surface of the silica gel is shown in Scheme 2.7.

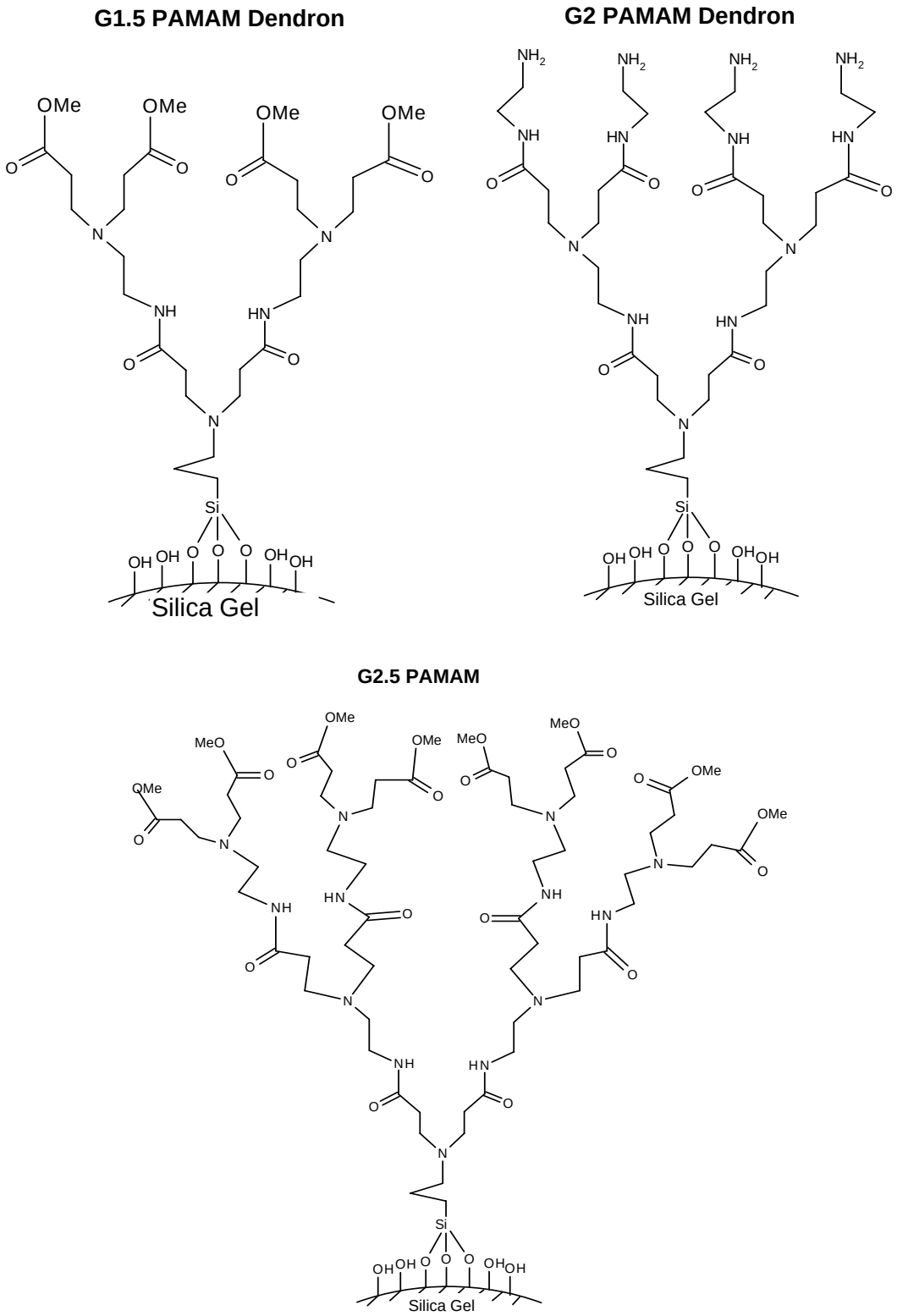
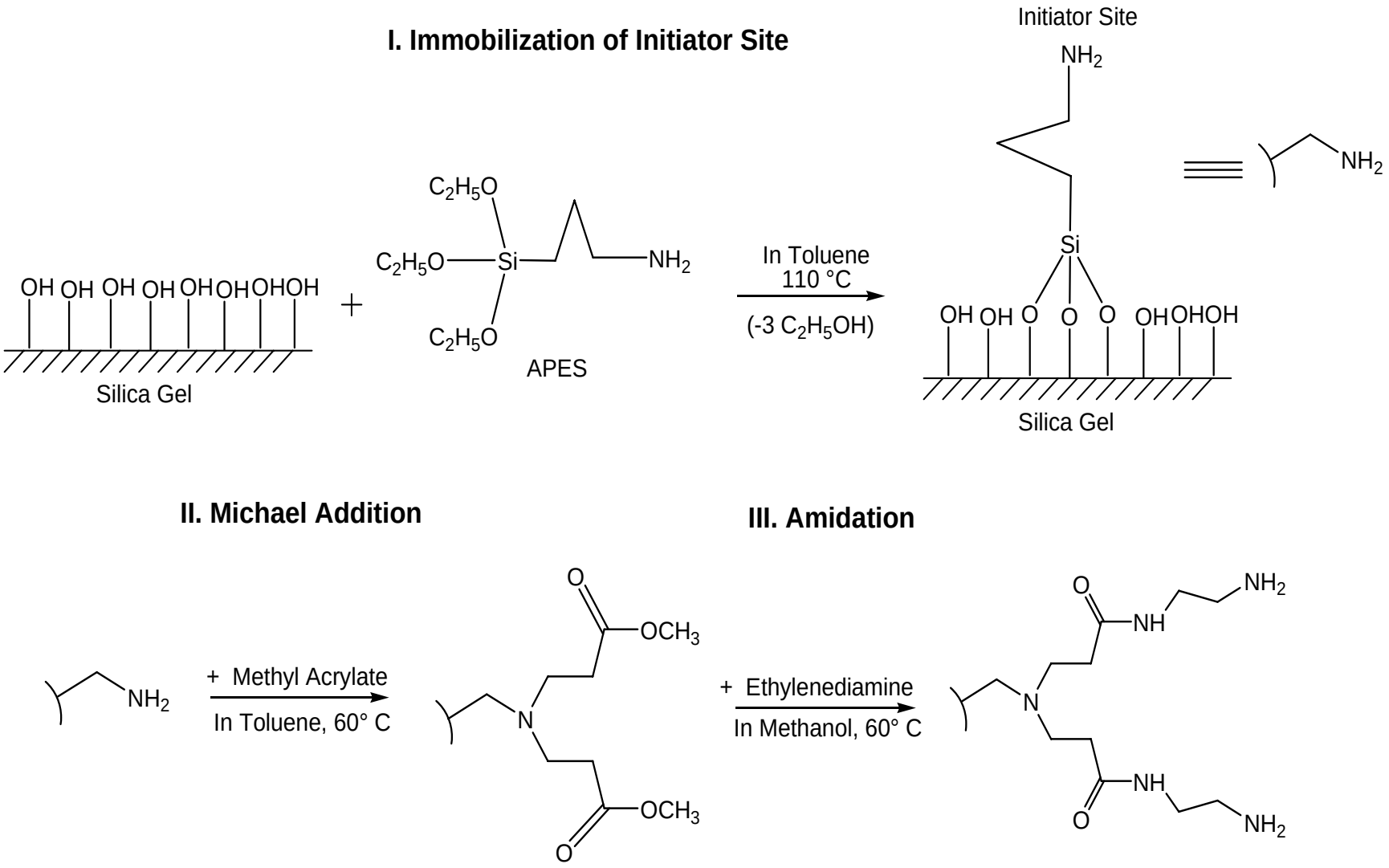


Figure 2.1 Theoretical illustration of the other higher generations synthesized.



Scheme 2.7 Representative reactions for the propagation of PAMAM dendrons in mesoporous spherical silica gel.

2.2 Experimental

2.2.1 Materials

PAMAM dendrons were propagated in ultra fine spherical silica gel (SiliCycle) with a particle size of 5 microns, pore diameter of 60 angstrom, and surface area of 500 m^2/g. The silica gel was dried in vacuo at 110 ^0C for 8 hours before use. Methyl acrylate purchased from Sigma-Aldrich and ethylenediamine from J.T. baker were distilled and refluxed over sodium. After distillation, the purity of methyl acrylate and ethylenediamine was verified by ^1H NMR. The toluene and methanol solvents obtained from Merck were dried using molecular sieves. Whatman 42 filter paper (retention size, 2.5 microns) purchased from United Scientific Equipment Pte Ltd was used in all the vacuum filtrations.

2.2.2 Procedures

2.2.2.1 Synthesis of G0 PAMAM Dendron

The grafting of an initiator site with amine functionality into the silica gel was achieved as follows: In a 250-milliliter flask (2-neck RBF) that contained 150 milliliters toluene, 10 grams of ultra fine spherical silica gel was added, and the mixture was constantly stirred in oil bath at 400 rpm speed. Upon reaching the set point temperature of 110 ^0C, 10 milliliters of 3-aminopropyltriethoxysilane (APES) was added immediately into the reactor using a syringe. The resulting mixture was refluxed for 42 hours at 110 ^0C. After the reaction, the solid was filtered and washed with the reaction solvent under vacuum filtration. The filter paper containing the solid was gently folded and was wrapped with another filter

paper that was also folded. Then the solid sample wrapped inside the filter paper was transferred into the soxhlet extraction apparatus and was extracted in toluene for half a day. After the extraction, the solid was collected from the filter paper and contained in a small glass bottle with a cap sealed with a filter paper. The sample was then dried in tube oven (at 110 ^{0}C, for about 8 hours) that is connected to a vacuum with a liquid nitrogen contained in a Dewar flask as an interphase to trap the excess organic phase and moisture. After drying, the tube oven was allowed to cool down first to 25 ^{0}C before taking the sample for analysis. This is to protect the solid from absorbing moisture from the atmosphere.

2.2.2.2 Synthesis of G0.5 PAMAM Dendron

The Michael addition of methyl acrylate to the amine terminal group generated half generation PAMAM dendron that was ester-terminated. This reaction was done in toluene at 60 ^{0}C.

Michael addition to generate G0.5 PAMAM dendron was carried out as follows: 10 grams of solid (silica gel obtained from the above reaction) was added into the 150 milliters toluene contained in the 250-milliliter flask (2-neck RBF). The resulting mixture was stirred in oil bath at 60 ^{0}C. Then 8 milliliters of methyl acrylate was added instantly into the reactor using a syringe when the reaction temperature had reached the set point. The mixture was refluxed for 48 hours with constant stirring. After the reaction, the solid was filtered and washed

with toluene under vacuum filtration. The filter paper that collected the solid was gently folded and was wrapped with another filter paper. Afterwards, the solid sample wrapped inside the filter paper was placed into the soxhlet extraction apparatus and was extracted in toluene for half a day. When the extraction was finished, the solid was transferred into a small glass container with a cap sealed with a filter paper. The sample was again dried in vacuo at 110 ^{0}C for 8 hours to remove the excess organic liquid phase. Before taking the sample from the tube oven for analysis, the oven was allowed to cool down first to 25 ^{0}C.

2.2.2.3 Synthesis of G1 PAMAM Dendron

The amidation of the half-generation ester-terminated PAMAM dendron with ethylenediamine generated full generation with amine terminal. This reaction was carried out in methanol at 60 ^{0}C.

The amidation of the ester terminal groups of the G0.5 PAMAM dendron was carried out as follows: Into a 250-milliliter flask (2-neck RBF) that contained 9.25 grams of solid (silica gel obtained from the previous reaction) in 150 milliliters of methanol, a large excess of ethylenediamine (37 milliliters) was added when the reaction temperature had reached 60 ^{0}C. The resulting mixture was constantly stirred in oil bath and was refluxed for 24 hours. After the reaction, the solid was filtered and washed with methanol under vacuum. Then the sample wrapped in filter paper was extracted in methanol for about 8 hours. After the soxhlet extraction, the sample contained in a small glass bottle with a

cap sealed with filter paper was dried in vacuo for another 8 hours at 110 ^{0}C to remove the excess organic liquid phase. Again, the tube oven was allowed to cool down first before taking the sample for analysis.

2.2.2.4 Synthesis of G1.5 PAMAM Dendron

The propagation of G1.5 PAMAM dendron was done as follows: In a 250-milliliter flask (2-neck RBF) containing 150 milliliters of toluene with 7 grams of solid (G1 PAMAM Dendron) previously obtained from the above reaction, 13 milliliters of methyl acrylate was added when the reaction temperature had reached 60 ^{0}C. The resulting mixture was constantly stirred in an oil bath and refluxed for 48 hours. When the reaction was complete, the solid was filtered and washed with toluene under vacuum filtration. Then the sample wrapped inside the filter paper was extracted with toluene using the soxhlet extraction apparatus for about half a day. Afterwards, the sample was dried in vacuo for 8 hours at 110 ^{0}C and taken out of the tube oven for analysis after it had cooled down to 25 ^{0}C.

2.2.2.5 Synthesis of G2 PAMAM Dendron

The amidation of the ester terminal groups of G1.5 PAMAM dendron with large excess ethylenediamine resulted to G2 PAMAM dendron. It was carried out in the following manner: 55 milliliters of ethylenediamine was added into the 250-milliliter flask (2-neck RBF) containing 6.79 grams of solid (G1.5 PAMAM Dendron) dissolved in 150 milliliters methanol when the reaction temperature had

reached 60 ^{0}C. The mixture was refluxed for only 24 hours while being stirred constantly in an oil bath using a magnetic stirrer with a speed of 400-rpm. After the reaction, the solid was filtered and washed with the reaction solvent under vacuum filtration followed by soxhlet extraction with methanol for 8 hours to remove the excess reagents used. Then the sample collected and contained in a small glass container with a cap sealed with filter paper was dried in vacuo at 110 ^{0}C for 8 hours. After cooling down the tube oven to room temperature, the sample was taken out for analysis.

2.2.2.6 Synthesis of G2.5 PAMAM Dendron

G2.5 PAMAM dendron was propagated by Michael addition of the amine terminal groups of G2 PAMAM dendron with methyl acrylate. The reaction was carried as follows: Into a 250-milliliter flask (2-neck RBF) containing 5.14 grams of solid (G2 PAMAM dendron) dissolved in 150 milliliters of toluene, 19 milliliters of methyl acrylate was added into the reactor which was constantly stirred at 60 ^{0}C, and the resulting mixture was refluxed for 48 hours. When the reaction was complete, the solid was filtered and washed with toluene under vacuum filtration after which a soxhlet extraction of the solid using toluene was done for half a day. Finally, the sample was dried in vacuo at 110 ^{0}C for 8 hours. The sample was only taken from the tube oven for analysis when it has cooled to 25 ^{0}C.

Continuing the synthesis further by repeating the two-step reactions – the Michael addition and amidation - will further generate higher generation PAMAM dendrons. For the amidation reaction, a large excess of ethylenediamine was necessary to drive the reaction to completion. Summarized in Table 2.1 are the actual amount of reagents used and their corresponding reaction conditions for each generation synthesized. In this work, PAMAM dendron was synthesized up to generation 2.5 only.

Table 2.1 Actual amount of reagents used and the reaction time of cleavage.

Generation	Solid (g)	Reagents (mL)	Solvents (mL)	Reaction Time (hr)
I. Initiator Site Grafting		APES	Toluene	
SiO2---->G0	10	10	150	42
II. Amidation Reaction		Ethylenediamine	Methanol	
G0.5---->G1	9.25	37	150	24
G1.5---->G2	6.79	55	150	24
III. Michael Addition		Methyl Acrylate	Toluene	
G0---->G0.5	10	8	150	48
G1---->G1.5	7	13	150	48
G2---->G2.5	5.14	19	150	48

The Michael addition was carried out for 48 hours to ensure complete reaction of the amine termini with methyl acrylate, which was fed in excess. At this length of time, which is longer than the Michael addition done by Bu Jie et. al., [10] the reaction is assured to be complete based on their kinetic study. On the other hand, amidation was carried out for 24 hours only. Based on the IR analysis of the G1 and G2 samples, the reaction was complete as shown by the

total disappearance of the ester peak that described a half-generation PAMAM dendron.

2.3. Characterizations and Interpretation of Results

2.3.1. Infrared Spectroscopy

A. Methodology

Before the IR analysis, each sample was dried in tube oven (at 110 ^{0}C, for 8 hours) connected to a vacuum with liquid nitrogen contained in a Dewar flask as interphase to trap the organic solvent and moisture that were removed from the sample by the vacuum pump.

Analysis of the silica gel with grafted PAMAM dendron was done at 100 scans and at 4 cm^{-1} resolutions using Diffuse Reflectance Infrared Fourier Transform (DRIFT) spectroscopy. Potassium bromide was scanned as background before running the sample in the instrument, and continuous nitrogen purging of the IR instrument was maintained before and during the scanning of the sample.

The (DRIFT) infrared spectrum of the silica-grafted-PAMAM dendron was recorded on a Bruker infrared spectrophotometer (Equinox 55). The original spectra measured were treated and analyzed using OPUS software, quantified in terms of the Kubelka-Munk unit.

B. Results and Discussions

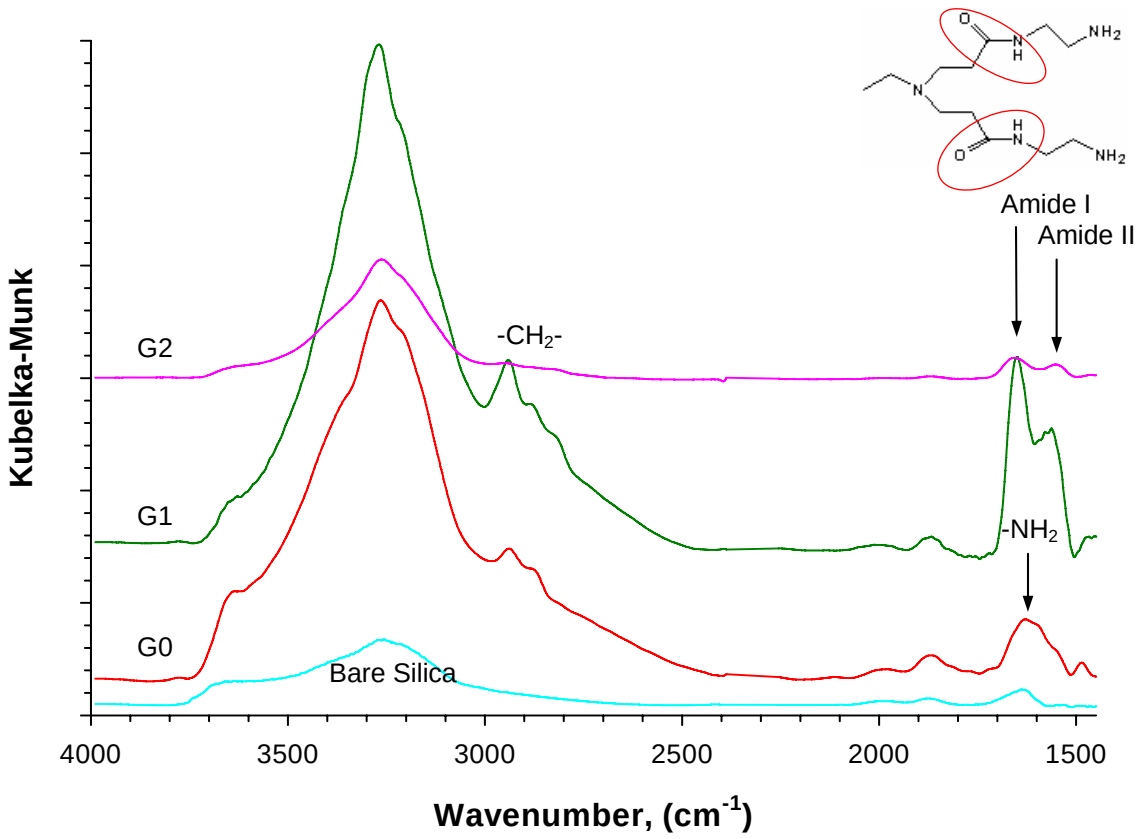


Figure 2.2 DRIFT spectra of the silica-grafted *amine* terminated PAMAM dendrons of increasing generations.

The initiator site (G0) to be used for the propagation of PAMAM dendrons was successfully immobilized onto the silica gel. The IR band (refer to Figure 2.2) at 1640 cm^{-1} corresponding to the N-H bending vibrations[8] of the primary amine, and the C-H bond stretching[8] found in the series of small peaks near 3000 cm^{-1} show the grafting of G0 into the silica gel. The peaks present in the spectrum of the amine-terminated initiator site are foreign to the bare silica gel as shown in Figure 2.2.

The presence of the surface immobilized PAMAM dendrons was also evident from the DRIFT spectra of the silica gel used in the synthesis. The spectra in the figure reveal two amide peaks (-CONH-) at 1550 and 1655 cm^{-1}, which are assigned to Amide II and I.[7] The Amide band I indicate the -C=O vibrations while Amide band II is attributed to the N-H bending vibrations in plane coupled with the valency C-N vibrations.[9] These amide bands are the characteristics of the amine-terminated full-generation PAMAM dendrons. For instance, only G1 and G2 contain these peaks. Moreover a series of small peaks near 3000 cm^{-1} corresponding to the C-H bond stretching[8] of the PAMAM dendrons are also featured in the spectrum.

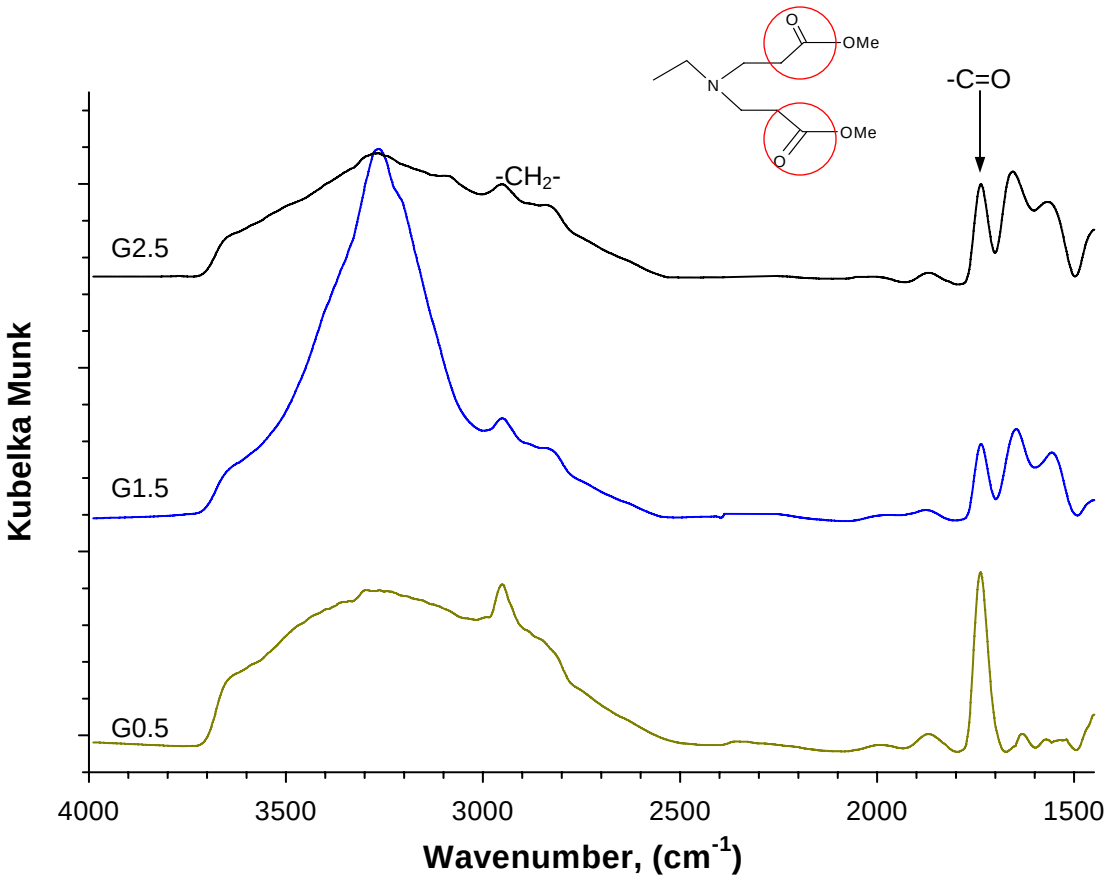


Figure 2.3 DRIFT spectra of the silica-grafted *ester* terminated PAMAM dendrons of increasing generations.

Similarly, the surface-immobilized half-generation PAMAM dendrons were demonstrated in the spectra (see Figure 2.3) by the peak at 1730 cm^{-1} corresponding to the -C=O vibration[8] of the ester functional group of the compound. Each spectrum of the half generations contains this distinct IR band. The series of small peaks near 3000 cm^{-1} corresponding to the C-H bond stretching[8] of the CH_2 functional group of the PAMAM dendrons still remain in the spectra, which is another evidence that the compound was successfully grafted into the silica gel.

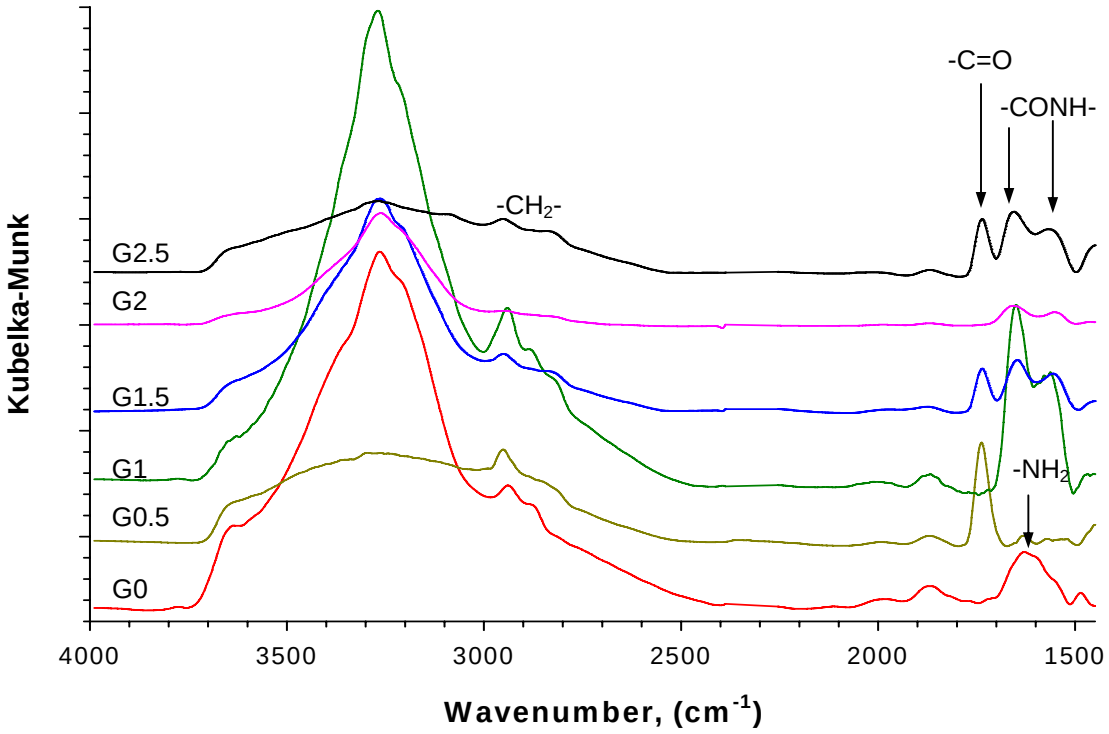


Figure 2.4 DRIFT spectra of the Michael addition and Amidation reactions of the silica-grafted PAMAM dendrons.

Figure 2.4 shows the propagation of PAMAM dendrons at different generations starting with the initiator site (G0) up to G2.5. Full-generation PAMAM dendrons are G1 and G2 while half generations are G0.5, G1.5 and G2.5.

Initially, the Michael addition of the amino reactive site of the initiator compound formed the generation 0.5. This is shown by the disappearance of the broad peak at 1640 cm^{-1} assigned to primary amine and the appearance of the –C=O vibration at 1730 cm^{-1} that is due to the ester functional group of the compound (G0.5). Likewise, the Michael addition of the full generation PAMAM

dendrons illustrated the appearance of the ester peak. This showed the conversion of the amine termini of the full generation into ester moieties during the reaction.

Amidation of the ester-terminated half-generation formed the amine-terminated full-generation PAMAM dendrons, which are described by the two-amide bands at 1550 and 1655 cm^{-1}.[7] The complete disappearance of the ester peak confirms the transformation of the ester group into amide functional groups indicating that the Amidation step is complete under the existing conditions.

It can be clearly noticed from the DRIFT spectra that the intensity of the signals for the amide band I and amide band II in the G2 decreased as compared to G1, despite the fact that both reactions have exactly the same reaction time. The possible explanation to this incidence is the cross-linking reaction that occurs more extensively in the higher generations. This issue will be illustrated further in the next section on thermogravimetry.

The said cross-linking reaction generates a structural defect that prevents further growth of the compound because the structure of the cross-link does not have any reactive site for the next reaction to take place, and Michael addition occurs only in the amine termini. Moreover, this cross-link defect cannot be distinguished from the desired compound having an ideal structure by simply using infrared spectroscopy because both compounds have amide functionality.

A more sophisticated analytical instrument like mass spectrometry is required to distinguish one from the other. However, the application of this analytical technique is only possible after the cleavage of the compound from the solid support.

2.3.2. Thermogravimetry (TGA)

A. Methodology

Before the TGA measurement, each sample was dried in tube oven (at 110 ^{0}C, for 8 hours), which was connected to a vacuum with liquid nitrogen contained in a Dewar flask as interphase to trap the organic solvent and moisture that were removed from the sample by the vacuum pump.

About 15 milligrams of the solid sample was loaded into the platinum TGA crucible. Initially, the TGA was set to isothermal setting for 5 minutes before heating to purge the unwanted gases inside the sample compartment. Using air with a flow rate of 200 milliliters per minute, the sample was heated from room temperature to 800 ^{0}C with constant temperature ramp of 5 ^{0}C per minute. When the final temperature was reached by the system, the instrument cooled down automatically using the same gas for heating the sample.

Thermogravimetric analyzer supplied by TA instruments of model SDT 2960 was used to analyze the silica-grafted-PAMAM dendrons. The instrument is

equipped with the Universal Analysis software to view the TGA curve of the sample after burning.

B. Results and Discussions

Table 2.2 TGA measurements of PAMAM dendron grafted on silica gel.

Synthesis Stage	Weight Grafted %*	Grafted Amt. (mg/g SiO2)	Theoretical. Amt. (mg/g SiO2)	Formula Weight
GO	11.83	118.3	118.3	58
GO.5	22.52	225.2	469.12	230
G1	26.37	263.7	583.34	286
G1.5	33.8	338	1284.98	630
G2	35.41	354.1	1513.42	742
G2.5	42.06	420.6	2916.71	1430

**Determined from the TGA weight-loss profile.*

The grafted amounts of PAMAM dendrons on the silica gel were determined from the thermogravimetric analysis. The results of the TGA measurements for each generation were summarized in Table 2.2. The calculation of the weight grafted is based on the formula[7] denoted as S. It is defined as the ratio of the grafted PAMAM dendrons per 1 gram of the bare silica gel. The difference in weight between 110 ^{0}C and 800 ^{0}C obviously accounts for the grafted PAMAM dendrons because at 800 ^{0}C all of the grafted organic phase have been burned and only the silica gel is left in the TGA crucible. That is also why the formula is divided by the weight at 800 ^{0}C. Moreover, the weight before 110 ^{0}C was not considered in the formula for it is mainly due to moisture adsorbed on the surface of the porous silica gel.

$$\textbf{S, (\%)} = \frac{(\text{Weight at } 110\ ^0\text{C} - \text{Weight at } 800\ ^0\text{C})}{\text{Weight at } 800\ ^0\text{C}} \times 100\%$$

The percent weight grafted due to the organic phase immobilized on the surface of silica gel increased from generation 0 to 2.5. This means that for every additional reaction in the silica-grafted compound, the dendron increased in size. Thus, the PAMAM dendron was successfully grown onto the silica gel. The TGA profile shows this clearly.

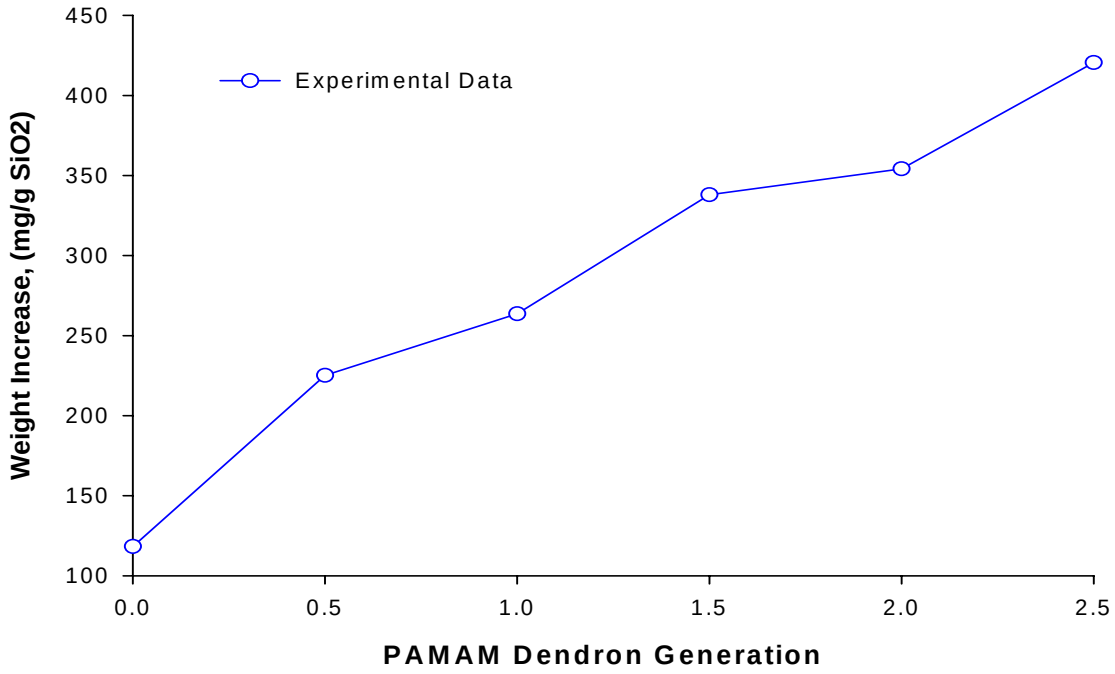


Figure 2.5 TGA profile of PAMAM dendrons.

It can be noticed from the TGA profile (see Figure 2.5) that as the generation increases, the weight increase of the organic phase declines for both Michael addition and amidation reactions. For instance G0 to G0.5 has a greater weight increase as compared to G1 to G1.5 and G2 to G2.5, while G0.5 to G1 has a greater weight increase also in contrast with G1.5 to G2. This explains that more side reactions occur in the higher generations forming structural defects. In

the research conducted by Peterson et al.,[11] it was mentioned that more structural defects are formed in the higher generations due to the many branches of the compound that cause crowding. As a result, the amount of propagation of PAMAM dendrons decreases as the generation increases.

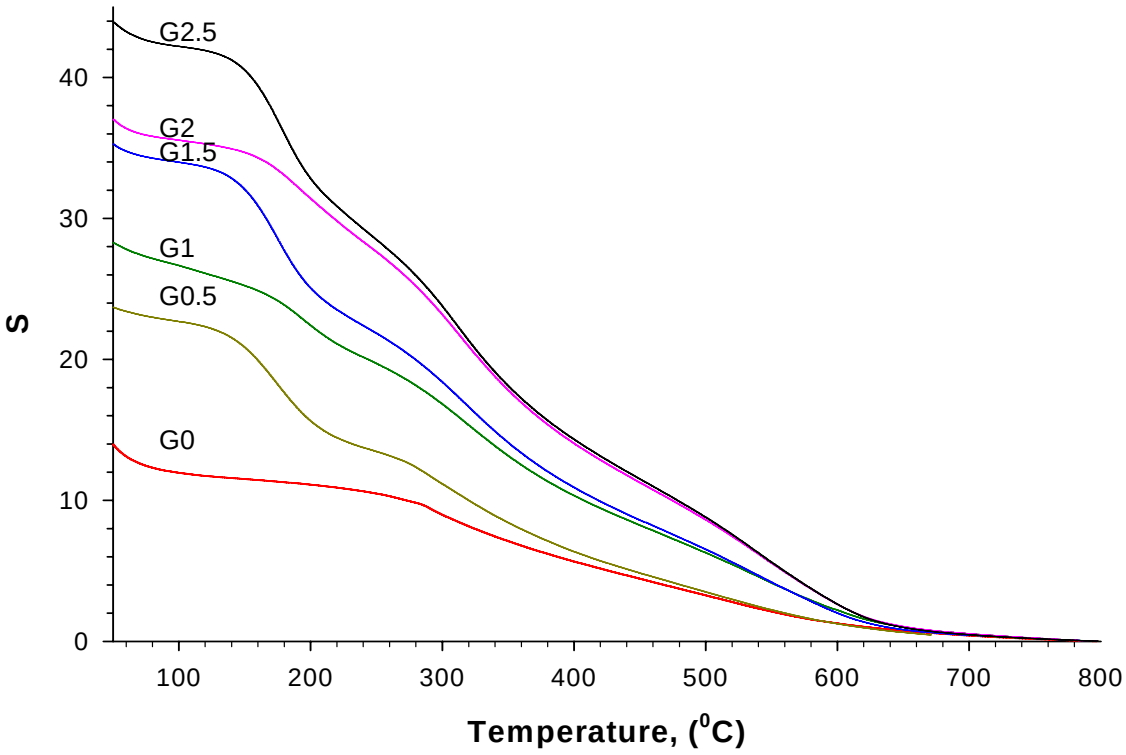


Figure 2.6 TGA analysis of PAMAM dendrons at different generations.

From the actual TGA measurement shown in Figure 2.6, the amidation step has a smaller weight difference as compared to the Michael Addition step although the infrared spectroscopy shows a complete disappearance of the ester peak (see Figure 2.4). Consider the weight difference of G2.5 from G2, G1.5

from G1 and G0.5 from G0. These regions have a greater weight differences as compared to G1 from G0.5, and G2 from G1.5.

This implies that a side reaction due to cross-linking is really happening in the amidation step, which significantly contributes to the low amount of propagation of PAMAM dendron. Theoretically, one ester terminal group in the silica-grafted compound should react only with one terminal amine group of the ethylenediamine monomer during the amidation reaction and leaving the other amine group of the monomer free for the next step that is Michael addition. However, when both of the amine terminal groups of the monomer react with adjacent ester terminal groups of the silica-grafted compound (see Figure 2.7) on the same step, cross-linking takes place and the expected amount of grafting decreases. Because of this cross-linking incident in the amidation step, the grafted amounts of PAMAM dendron are smaller than the expected value (see Table 2.2).

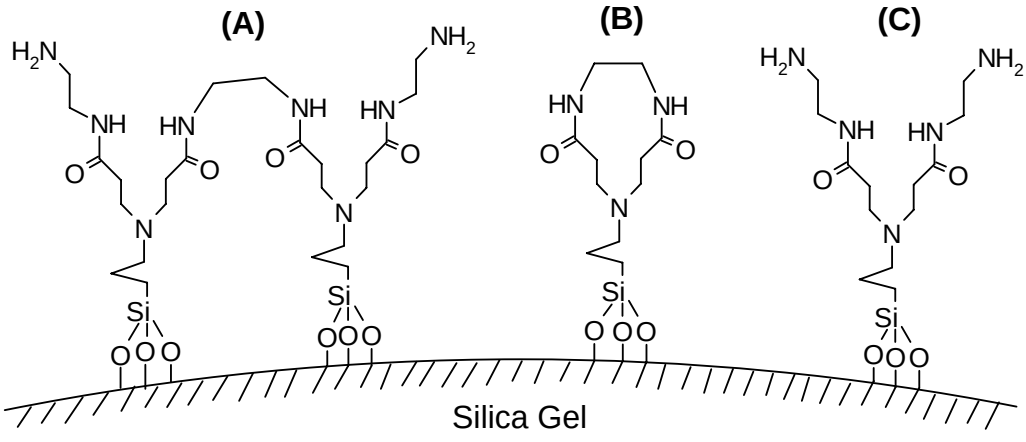
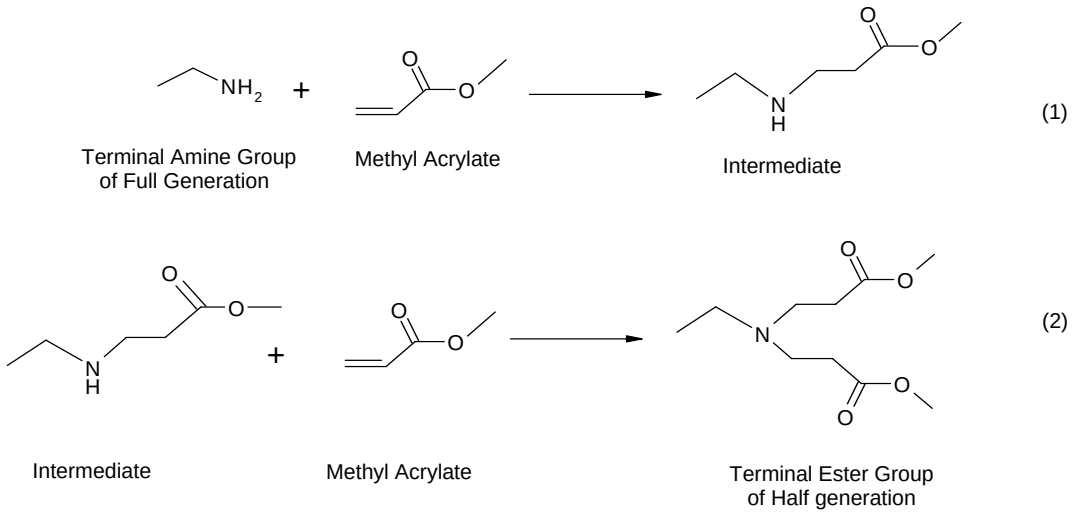


Figure 2.7 (A) Shows cross-linking between adjacent amine group from two neighboring branches[10] (B) Internal cross-linking also known as intramolecular cyclization[11] (C) Ideal structure of G1 PAMAM dendron with no cross-linking.

The Michael addition has a greater weight difference as compared to the amidation reaction, (see Table 2.2). This implies that a side reaction also occurs during the Michael addition, and the previous measurement by infrared spectroscopy fails to determine this incident because the product of the side reaction also contains an ester functional group that gives exactly the same IR band as the product.

This supports the findings of Bu Jie et al.[10] that a side reaction occurs in spite of the complete Michael addition as shown in the result of their kinetic study using infrared spectroscopy. In Scheme 2.8 that is proposed by them, the ester group in the intermediate product creates a steric hindrance for the incoming monomer (methyl acrylate). Thus, the new compound has missing branch/branches. As such, this phenomenon is called incomplete Michael addition. When this occurs, the amount of grafting decreases as shown in the experimental results (see Table 2.2).



Scheme 2.8 (1) Incomplete Michael addition shows the formation of an intermediate; (2) Complete Michael addition of the amine terminal group to form the ideal structure of the half-generation PAMAM dendron having two ester groups at the terminal ends. *Proposed by Bu Jie, et al.* [7]

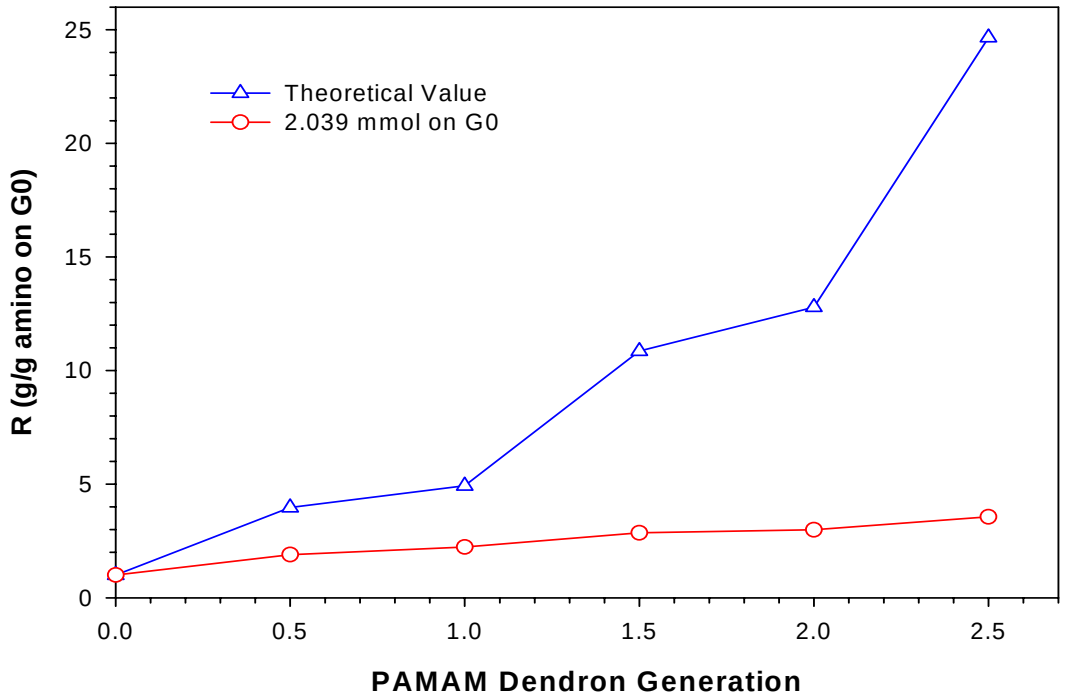


Figure 2.8 Synthesis result compared to the theoretical amount of grafting the PAMAM dendron onto the silica gel.

The efficiency of the synthesis was compared based on the theoretical value of R where R is the ratio of the grafted amount to the initial amino group content. Despite the increase in the R-values of the synthesis for each generation, experimental values are still smaller than the theoretical (refer to Figure 2.8). This elucidates the low efficiency of the synthesis method, particularly in the higher generations as shown by the increasing gap between the experimental and theoretical lines in the plot. The earlier works of Tsubukawa, et al.[1] also exhibit a low efficiency in the synthesis of the compound. The values of R are listed in Table 2.3.

Table 2.3 Computation of the R Values.

Synthesis Stage	Theoretical *R	Experimental R
GO	1.00	1.00
GO.5	3.97	1.90
G1	4.93	2.23
G1.5	10.86	2.86
G2	12.79	2.99
G2.5	24.66	3.56

**Ratio of the grafted amount to the initial amino group content*

In this experiment, the initial amino group content of the silica gel used to propagate PAMAM dendrons is 2.039 millimoles, as determined by the TGA measurement. This amount is really difficult to control when exactly the same reaction is done because the silylation reaction is non-uniform[3] as mentioned earlier. The experiment has proven this.

With a high starting density of the amino group in the initiator site (2.039 millimoles), the higher generations are more likely to exhibit a crowding effect and decrease the amount of propagation. This explains why the intensity of the signal in the IR measurement for G2 PAMAM dendron is low as compared to that of the G1 PAMAM dendron (see Figure 2.2). Although some structural defects are formed in the second generation, the propagation of G2.5 was achieved in the experiment as shown by the TGA and FTIR analyses. This proves that further grafting of the compound is still feasible despite the defects that were formed in the previous reactions.

2.4 Conclusion

Although the PAMAM dendron of different generations was successfully grafted into the silica gel, the propagation of the compound was not achieved theoretically as shown in Table 2.2. The possible reasons for the low efficiency of the synthesis are the incomplete Michael addition due to steric hindrance and the cross-linking reactions in the amidation step. The results showed that the problems that contribute to the formation of the structural defects of the compound are more serious in the higher generations. And between the two synthesis problems mentioned, the cross-linking reaction is the main cause of the small amount of grafting in the experimental result as shown in the TGA profile by the lesser weight increase in the formation of the full-generation PAMAM dendrons (G0.5 to G1 and G1.5 to G2) as compared to the formation of the half-generation.

2.5 References

1. Tsubukawa, N.; Ichioka, H.; Satoh,H.; Hayashi, S.; Fujiki, K. ***React. Funct. Polym.*** 1998, 37, 75-82.
2. Nguyen, V.; Yoshida, W.; Cohen, Y. ***J. Appl. Polym. Sci.*** 2003, 87, 300-310.
3. Yoshida, W.; Castro, R.P.; Jou, J.D.; Cohen, Y. ***Langmuir***. 2001, 17, 5882.
4. Blitz, J.P.; Murthy, R.S.S.; Leyden, D.E. ***J. Am. Chem. Soc.*** 1987, 109, 7141.
5. Van Der Voort, P.; Vansant, E.F. ***J. Liq. Chromatogr. R. T.*** 1996, 19, 2723.
6. Hunsche, A.; Goerl, U.; Mueller, U.; Knaack, M.; Goebel, T. ***Kaut. Gummi Kunstst.*** 1997, 50, 881.
7. Bu, J.; Li, R.; Quah, C.W.; Carpenter, K.J. ***Macromolecules***. 2004, 37, 6687-6694.
8. Pavia, D.L.; Lampman, G.M.; Kriz, G.S. ***Intoduction to Spectroscopy***. 3rd edition.
9. Nikolic, L.; Skala, D.; Nikolic, V.; Stamenkovic, J.; Babic, D.; Stojanovic, S.L. ***J. Appl. Polym. Sci.*** 2004, 91, 387-395.
10. Li, R.; Bu, J. ***Korean J. Chem. Eng***. 2004, 21, 1, 98-103.
11. Peterson, J.; Ebber, A.; Allikmaa, V.; Lopp, M. ***Proc. Estonian Acad. Sci. Chem.*** 2001, 50, 3, 156-166.
12. Tomalia, D.A.; Baker, H.; Dewald, J.; Hall, M.; Kallos, G.; Martin, S.; Roeck, J.; Ryder, J.; Smith, P. ***Polym. J.*** 1985, 17, 1, 117-132.

Chapter 3: Cleavage of 3-(diethylenetriamino)propyl from the Silica Gel

3.1 Introduction

A commercial sample, 3-(diethylenetriamino)propyl - functionalized silica gel, was used in the reaction to test the effectiveness of the cleavage method before using the solid-phase synthesized dendritic macromolecules – PAMAM dendrons. The model compound that is also grafted onto the silica gel was selectively chosen for the cleavage because it has an amine terminal group that is similar to the terminal groups of the full generation PAMAM dendrons.

In the past, scientists had proven that organosilanes could be oxidatively cleaved from the silica gel support through the silicon-carbon bond.[1-3] One approach is to use hydrogen peroxide in the presence of potassium fluoride. Since the organic phase bonded to silica gel through silicon oxygen bonds are organosilanes with one or more electron-withdrawing oxygens, the hydrogen peroxide in the presence of potassium fluoride can oxidatively cleave the organic phase like the silica-grafted PAMAM dendrons from the solid support.

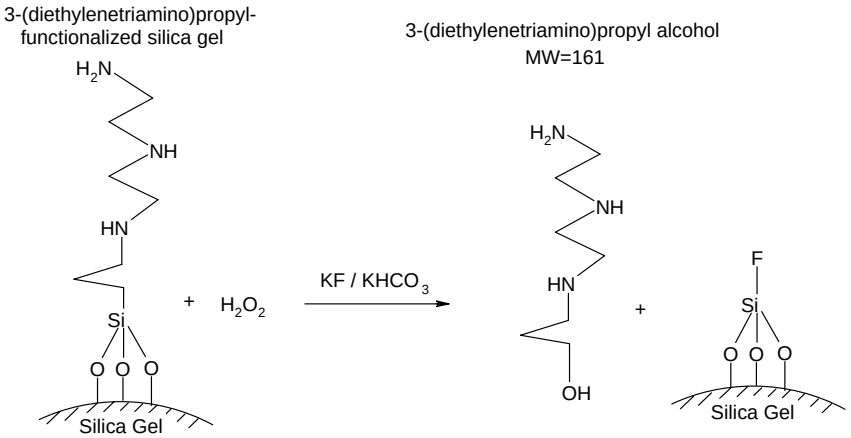
Furthermore, the reaction is quite universal because it works with most organosilanes that have one or more electron-withdrawing substituents like oxygen.[4] Functional groups like ester are able to withstand the reaction because the condition is mild rather than acidic or basic. Moreover, the reaction forms very stable and easily characterizable end products like alcohols. In acidic and

basic conditions, cleavage of the organic phase from silica gel is also possible but it leads to very complicated products as confirmed by both H NMR and thin-layer chromatography.[4]

Chemical cleavage of the bonded organic phase from the surface of the silica gel followed by spectroscopic or mass characterizations is a powerful method for structural analysis of the solid-phase synthesized compound. Tingli, et al.[4] conducted an earlier study regarding the cleavage of the different stationary phases grafted onto the silica gel. The stationary phase mentioned in their study is referred to as the organic phase. The Pirkle chiral leucine selector was one of the stationary phases used in the experiment. It has amide functionality similar to that of the full generation PAMAM dendron.

After the cleavage, the sample was analyzed by tandem (MS-MS) mass spectrometry with electrospray ionization technique. The significant advantages of using ESI-MS include its high sensitivity and soft ionization.[5] To confirm the results of the ESI MS, the cleavage sample was sent for further analysis in the Time-of-Flight (TOF) mass spectrometry. The TOF-MS is tolerant to impurities, and it produces predominantly single charged ions, allowing for the determination of the direct mass number of polymers.[6] Furthermore, the infrared spectroscopy was used to support the results of the mass analyses by finding out the important molecular vibrations of the different functional groups that are present in the target compound, 3-(diethylenetriamino)propyl alcohol.

Finally, the silica gel after cleavage was characterized by thermogravimetry (TGA), elemental analysis (EA), and infrared spectroscopy. The efficiency of the cleavage method was computed from the TGA and EA measurements before and after the cleavage of the compound. The theoretical illustration of the cleavage reaction is shown in Scheme 3.1.



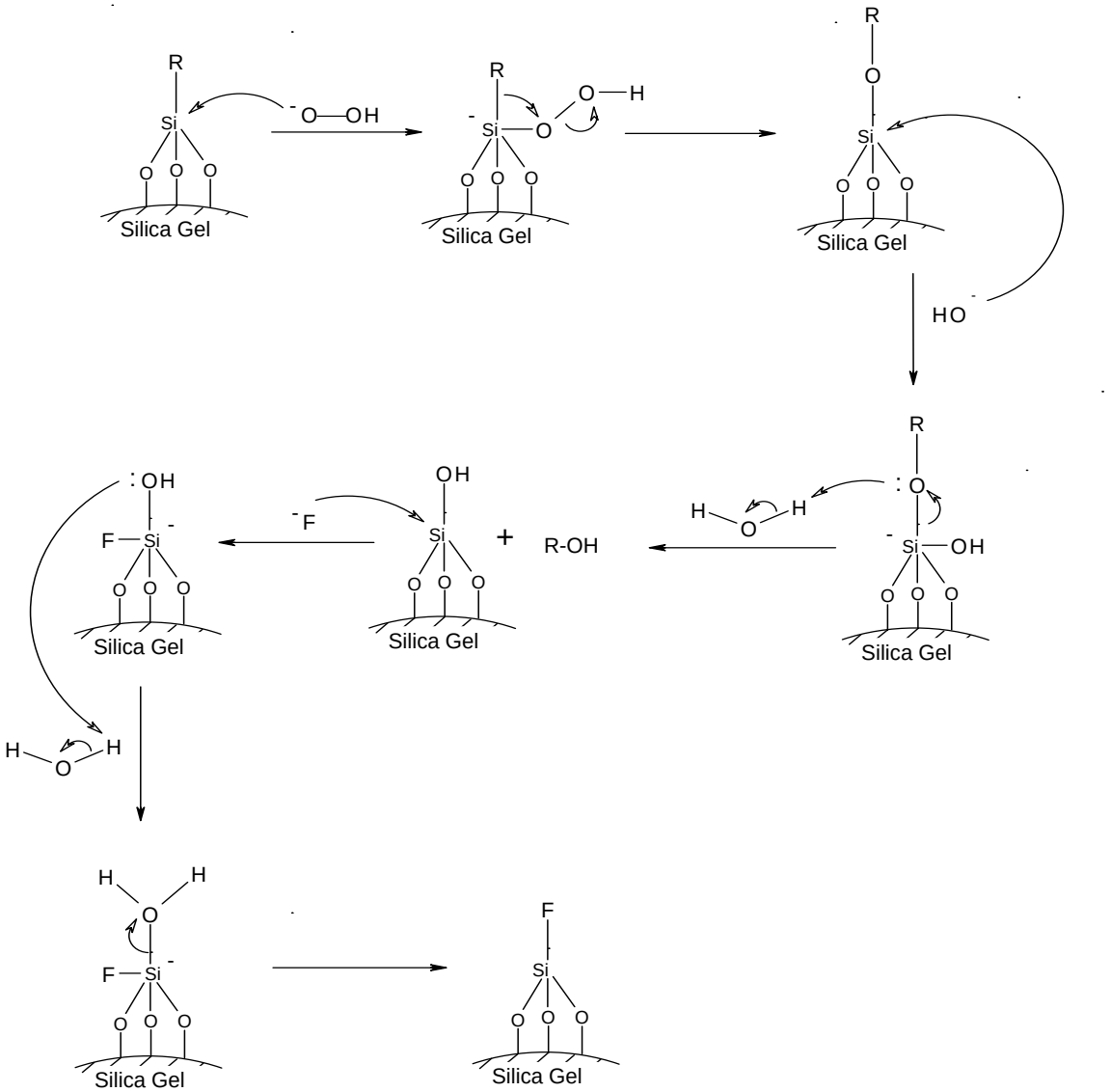
Scheme 3.1 Cleavage 3-(diethylenetriamino)propyl from silica gel.

The mechanism is described in Scheme 3.2. Hydrogen peroxide is first deprotonated by bicarbonate, and then it initiates the cleavage reaction by a nucleophilic attack to the silicon atom in the organic phase grafted onto the silica gel. This results to a negatively-charge complex. In order for the silicon to gain neutrality, the alkyl group attached to it undergoes migration onto the peroxide linkage, displacing the hydroxide group.

Hydrolysis of the resulting silicon-oxygen bond takes place when a nucleophile from hydrogen peroxide attacks the silicon atom, making it negatively-charge and the oxygen of the alkoxy group attracts a proton from a

water molecule and leaves as an alcohol. This end product that is an alcohol is usually very stable and easily characterizable.

The silicon atom is then attacked by the fluoride ion, causing the attached hydroxyl group to gain a proton and leaves as a water molecule. As a result, a strong silicon-fluorine bond is formed, preventing any possibility of the backward reaction.



Scheme 3.2 Reaction mechanism of the cleavage.

3.2 Experimental

3.2.1. Materials

The 3-(diethylenetriamino)propyl - functionalized silica gel purchased from Sigma-Aldrich was kept in a dry cabinet at constant temperature of 20 ^{0}C while the 30% aqueous hydrogen peroxide supplied by Kanto Chemical Co., Inc. was stored in the fridge at low temperature. The solvents used were purchased from either Sigma-Aldrich, J.T. Baker, or Merck. Potassium bicarbonate supplied by Sigma-Aldrich and potassium fluoride from Riedel-de Hgen were also kept in the dry cabinet to protect them from absorbing moisture from the surrounding atmosphere. The filter paper, Whatman 42 (retention size, 2.5 microns), from United Scientific Equipment Pte Ltd was used in the filtration.

3.2.2. Procedures

The cleavage of 3-(diethylenetriamino)propyl from surface of silica gel (refer to Scheme 3.1) was done in three different solvents like methanol, THF/methanol and water using hydrogen peroxide (H_2O_2) in the presence of potassium fluoride (KF) and potassium bicarbonate ($KHCO_3$). This was carried out as follows: Into a 100 ml flask that contained 1 gram of solid, 3-(diethylenetriamino)propyl - functionalized silica gel), dissolved in solvent (30 ml), potassium fluoride (10 equivalents to the organic phase) and potassium bicarbonate (10 equiv.) were added, and the resulting mixture was stirred in oil bath at 60 degrees Celsius. When the reaction temperature reached nearly the set point, 30% aqueous hydrogen peroxide (10 equiv.) was added using the

micro pipette and then the mixture was refluxed for 12 hours with constant stirring using a magnetic stirrer with a speed of 400 rpm. The reaction conditions of the cleavage were summarized in Table 3.1.

Table 3.1 Summary of cleavage reaction conditions.

Reaction Time	12 hours
Temperature	60 ^{0}C
Reagents	1.) 30% aq. H_2O_2 - 10 equiv. to the organic phase 2.) Potassium Carbonate - 10 equiv. to the organic phase 3.) Potassium Flouride - 10 equiv. to the organic phase
Solvents: (1) Methanol (2) THF/Methanol (3) Water	30 milliliters per 1 gram of 3-(diethylenetriamino)propyl - functionalized silica gel

After the reaction, the solvent was removed under vacuum using the rotary evaporator. Then the residue was dissolved in small amount of solution containing 10% methanol in dichloromethane and transferred to the glass column packed with silica gel for purification. After washing with 10% methanol in dichloromethane using the column, the filtrate was concentrated to yield the desired compound, which was analyzed by infrared spectroscopy and mass spectrometry.

The silica gel inside the column was retrieved using the pipette filler. It was then washed with the reaction solvent followed by large amount of de-ionized water to remove the inorganic components adsorbed on the surface of the silica gel. After washing, the solid support was dried in vacuo at 110 ^{0}C for 8 hours to remove the trace amount of solvent and moisture present on the

surface. Finally, the silica gel was analyzed by infrared spectroscopy, thermogravimetry, and elemental analysis.

3.3 Characterizations and Interpretation of Results

3.3.1. Infrared Spectroscopy

A. Methodology

a.) **Silica Gel**. Before the IR measurement, each solid sample was dried in tube oven (at 110 ^{0}C, for 8 hours), which was connected to a vacuum with liquid nitrogen contained in a Dewar flask as interphase to trap the organic solvent and moisture that were removed from the sample by the vacuum pump.

The analysis of silica gel was done at 40 scans having 4 cm^{-1} resolutions using the Digilab Excalibur FTIR. Initially, potassium bromide was pressed into a translucent disc using a hydraulic press and was run into the instrument as the background. Subsequently, the sample was prepared by mixing the silica gel with potassium bromide and was pressed into a translucent disc also under vacuum suction. The spectra were quantified in terms of absorbance unit.

b.) **Cleavage compound**. A translucent disc of potassium bromide was made first using the hydraulic press; and then the cleavage compound, a pale yellow viscous liquid, was pasted on the center of the disc. The sample

was run in the instrument at 40 scans and 4 cm^{-1} resolutions. Potassium bromide was also scanned as the background before the sample measurement. The spectra were quantified in terms of absorbance units.

B. Results and Discussions

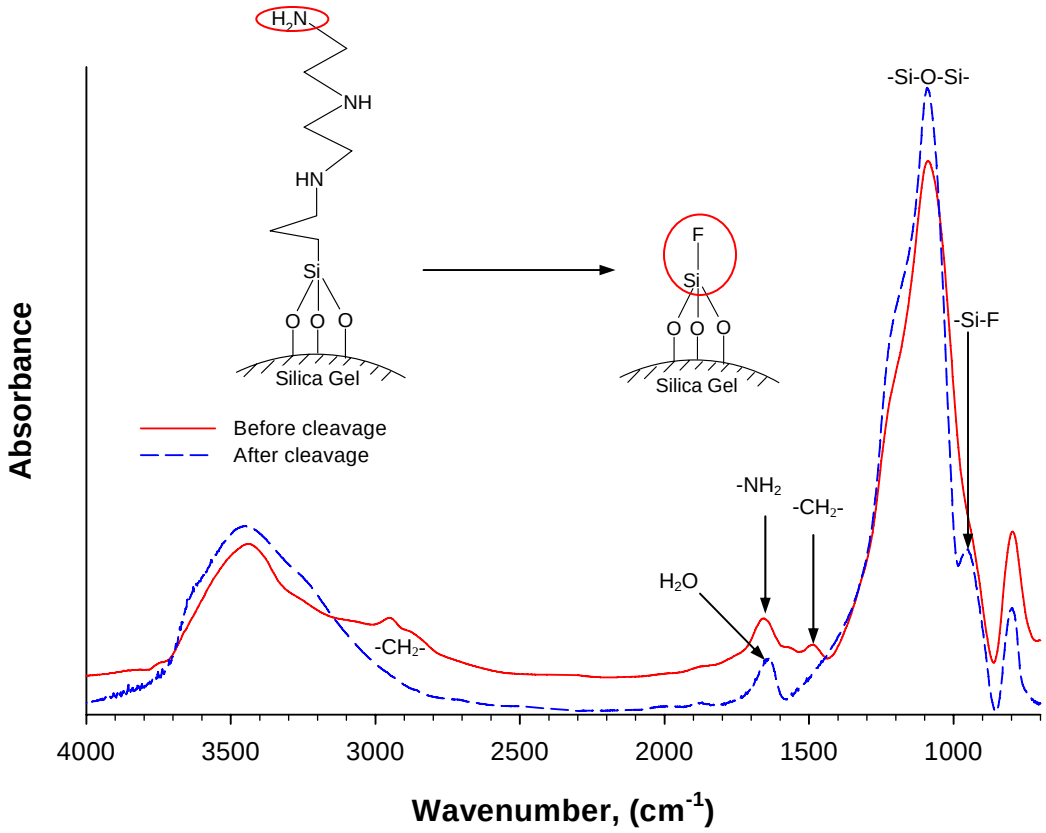


Figure 3.1 IR spectrum of the silica gel before and after cleavage of 3-(diethylenetriamino)propyl in *methanol.*

The 3-(diethylenetriamino)propyl was successfully cleaved from the silica gel as shown in the IR spectrum (see Figure 3.1) by the disappearance of the N-H bending vibrations[7] at 1640 cm^{-1}, which is due to the amine functional group of the compound. Another peak appeared in this region after the cleavage of the compound from the silica gel support. This new peak that is very close to the N-H

bending vibrations of the amine group is attributed to moisture. Water molecules can easily adsorbed into the surface of the porous silica gel since the organic phase has been cleaved from its surface. The peak at 1635 cm^{-1} is only assigned to the OH stretching vibration of the water molecules and is not due to the OH group of the cleavage compound because there is no evidence of the CH_2 stretching vibration in the spectrum of the silica gel after the cleavage. To illustrate further, the 3-(diethylenetriamino)propyl that is immobilized onto the surface of the silica gel is described by the different CH_2 vibrations of the compound. These are the C-H bond stretching[7] indicated by the series of small peaks near 3000 cm^{-1} and C-H bending vibrations[7] at 1475 cm^{-1}. After the cleavage of the compound, the series of CH_2 bands disappeared. Also, the silica gel has been washed thoroughly with water and methanol alternately followed by high temperature drying in tube oven that is connected to a vacuum pump. Thus, the compound is completely removed from the surface of the silica particles after the cleavage.

Another IR band that can be observed in the spectrum is the Si-O-Si stretching vibration[7] of the silica gel at 1080 cm^{-1}. This is the strongest band that is both seen in the spectrum of the silica gel before and after cleavage of the compound. Finally, the stretching vibration of the silicon-fluorine bond[7] is shown at 950 cm^{-1}. This peak is not apparent in the spectrum of the 3-(diethylenetriamino)propyl – functionalized silica gel. The fluorine content of the silica gel after the cleavage was determined by elemental analysis as

summarized in Table 3.3. And for the cleavage of the compound in THF/methanol and in water, the same results were obtained in the infrared spectroscopy (refer to Figures 3.2 and 3.3).

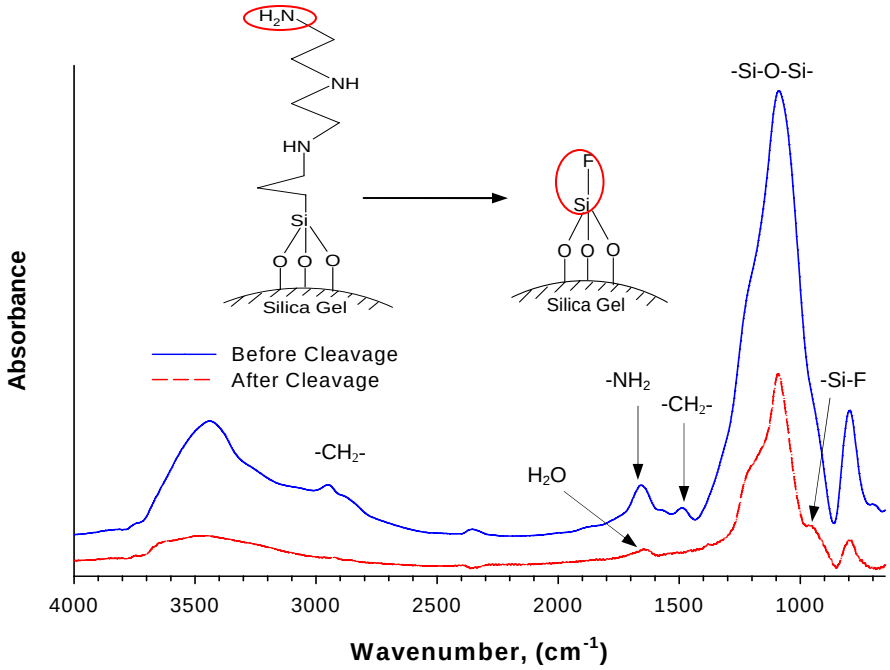


Figure 3.2 IR spectrum of the silica gel before and after cleavage of 3-(diethylenetriamino)propyl in *THF/methanol.*

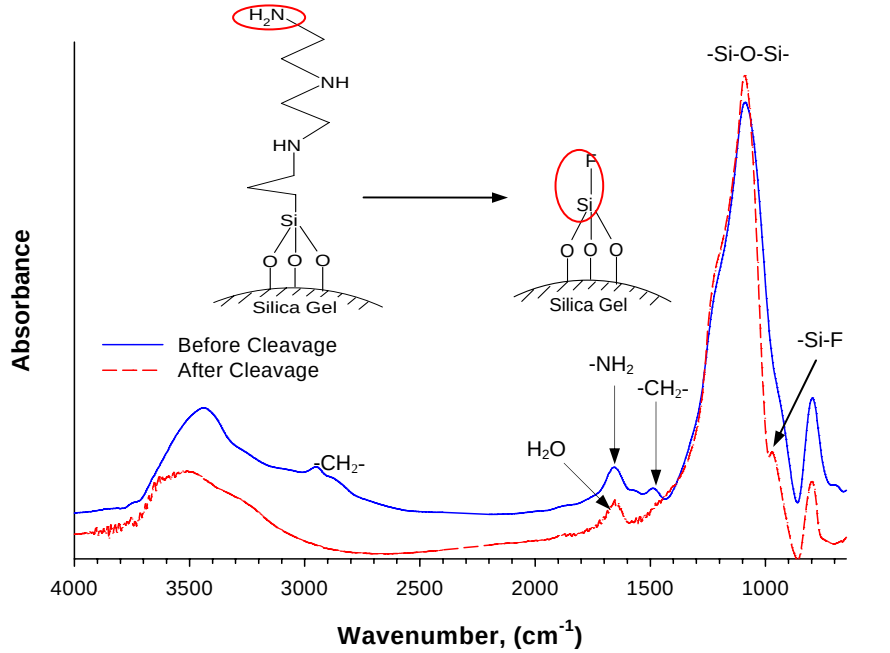


Figure 3.3 IR spectrum of the silica gel before and after cleavage of 3-(diethylenetriamino)propyl in *water.*

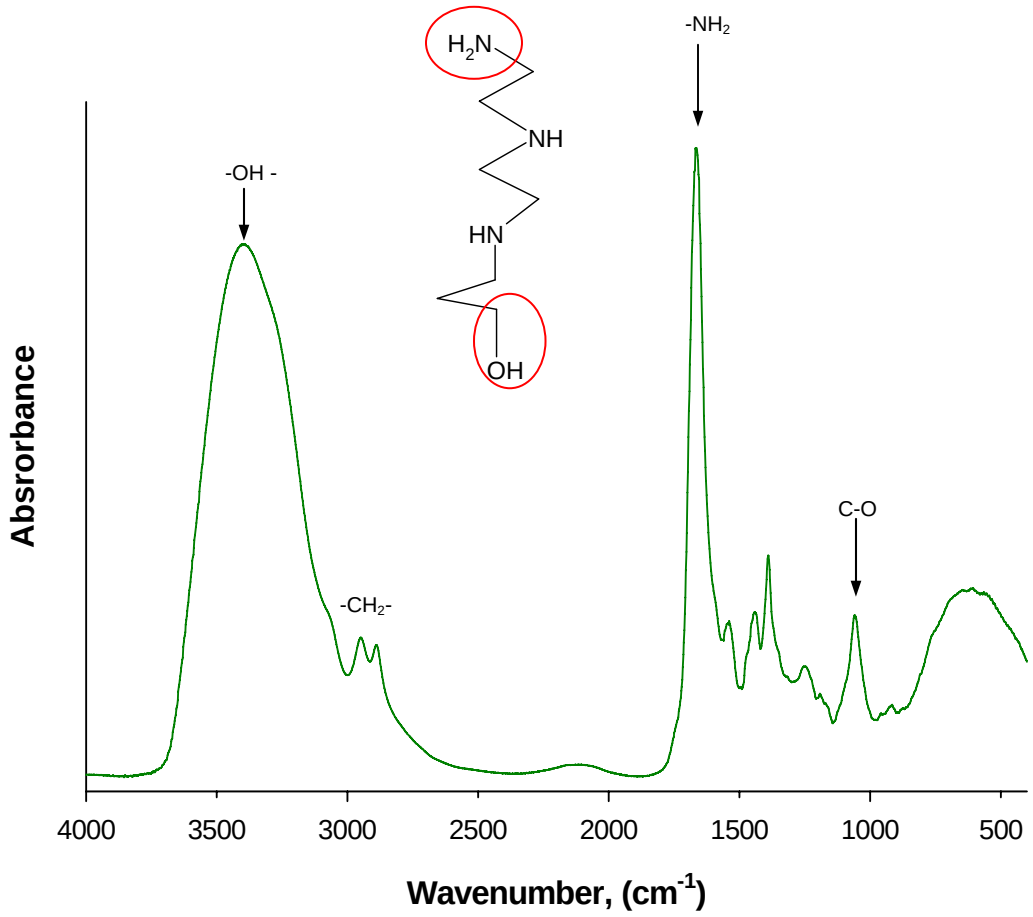


Figure 3.4 IR spectrum of the 3-(diethylenetriamino)propyl alcohol after cleavage in *methanol.*

The IR spectrum of the liquid phase (Figure 3.4) undoubtedly demonstrates the presence of the compound, 3-(diethylenetriamino)propyl alcohol, in the cleavage sample by checking the important molecular vibrations. The N-H bending vibration[7] of the compound's amine functional group at 1640 cm^{-1} is shown at a very high intensity. The C-H bond-stretching vibrations[7] of the CH_2 functional groups indicated by the small peaks near 3000 cm^{-1} strongly support the presence of the organic compound in the liquid phase.

The broad peak at 3400 cm^{-1} illustrates the OH functional group of the compound, which is due to the stretching vibrations[7]. It is very unlikely that this peak is due to the OH group of methanol since this solvent has been completely evaporated during the experiment. Further the C-O stretch[7] is clearly seen at 1060 cm^{-1}, which is a characteristic of a primary alcohol. This confirms that after the cleavage of the compound, the product formed is an alcohol as shown by the OH and C-O bands in the IR analysis. Similarly, this proves that a C-OH bond is formed in the compound replacing the Si-C bond after the cleavage. The same results were also obtained for the cleavage of the compound in THF/methanol and in water. These are shown in Figures 3.5 and 3.6.

Since the IR only shows the vibrations of the different functional groups of the compound, powerful analytical technique such as mass spectrometry has been used also to determine the molar mass of the compound to confirm its structure, and the mass analysis in section 3.3.4 gives the correct molecular weight of the compound in the spectrum. This shows that the IR vibrations assigned to the different functional groups are accurate evidence of the compound. Thus, both IR and mass analysis agree that the compound is present in the cleavage sample.

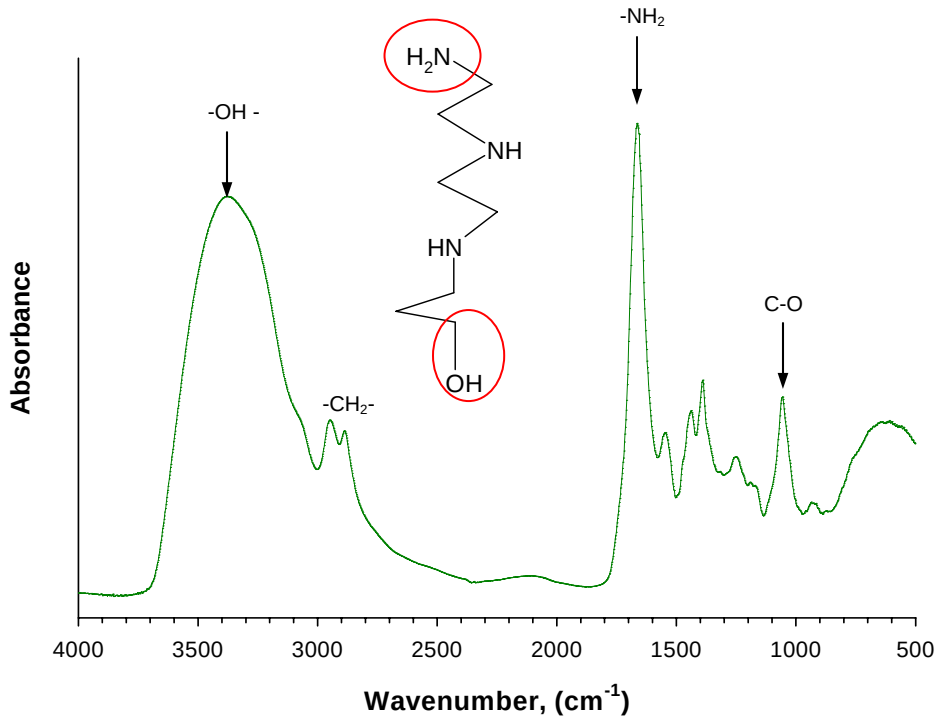


Figure 3.5 IR spectrum of the 3-(diethylenetriamino)propyl alcohol after cleavage in *THF/methanol.*

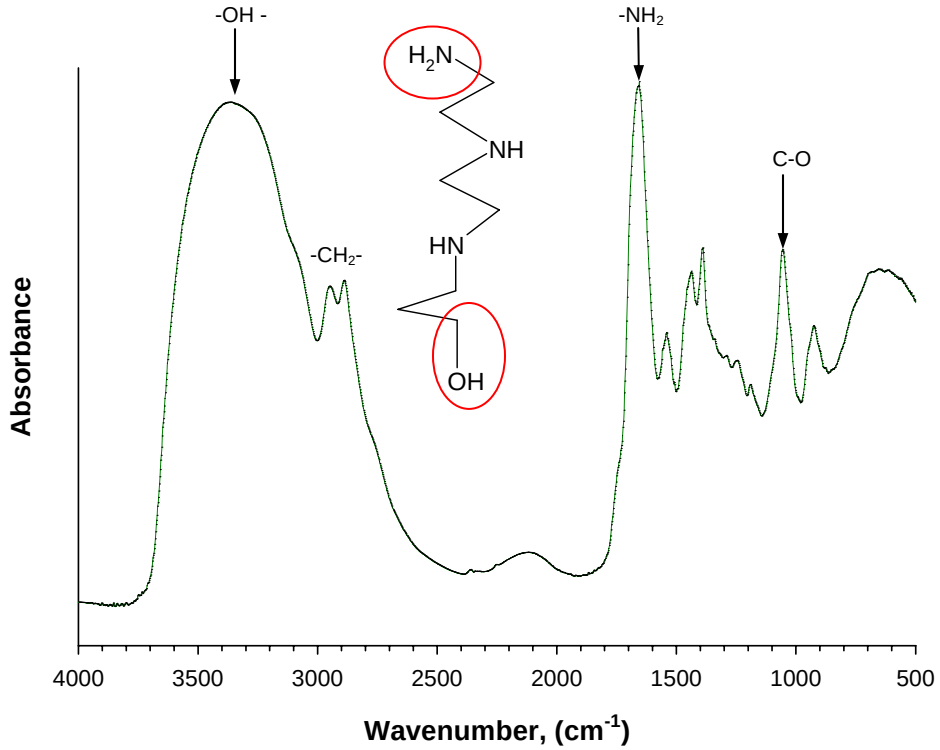


Figure 3.6 IR spectrum of the 3-(diethylenetriamino)propyl alcohol after cleavage in *water.*

3.3.2. Thermogravimetry

A. Methodology

Before the TGA analysis, each solid sample was dried in a tube oven (at 110 ^{0}C, for 8 hours), which was connected to a vacuum with liquid nitrogen contained in a Dewar flask as interphase to trap the organic solvent and moisture that were removed from the sample by the vacuum pump.

To compare accurately the result of the TGA measurements before and after cleavage, the same method was used in the instrument to analyze the samples. About 15 milligrams of the solid sample was loaded into the platinum TGA crucible. Initially, the TGA was set to isothermal setting for 5 minutes before heating to purge the unwanted gases inside the sample compartment. Using air with a flow rate of 200 milliters per minute, the sample was heated from room temperature to 800 ^{0}C with constant temperature ramp of 5 ^{0}C per minute. When the final temperature was reached by the system, the instrument cooled down automatically using the same gas for heating the sample.

Thermo-gravimetric analyzer supplied by TA instruments of model SDT 2960 was used to analyze the silica gel. The instrument is equipped with the Universal Analysis software to view the TGA curve of the sample after burning.

B. Results and Discussions

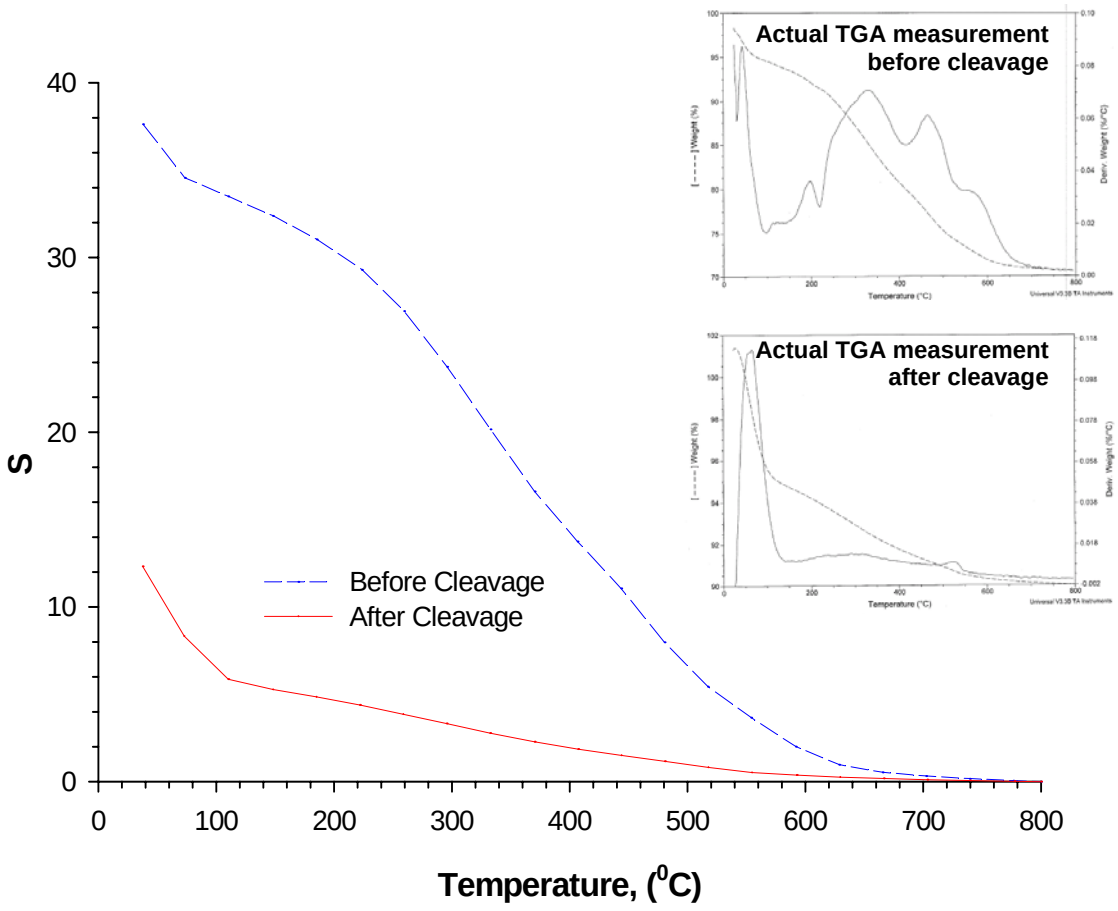


Figure 3.7 TGA profile of silica gel before and after cleavage of 3-(diethylenetriamino)propyl - functionalized silica gel in *methanol.*

The TGA measurement (see Figure 3.7) shows the success of the cleavage of 3-(diethylenetriamino)propyl from the silica gel. Initially, the percent weight grafted due to the organic phase immobilized on the surface of the silica gel was 33.5%. After the cleavage, percent weight grafted decreased to 5.8%. Both readings were taken at 110 ^{0}C in order not to consider the weight of the moisture content of the porous silica gel. In other words, the weight loss prior to 110 ^{0}C is not due to the grafted organic phase that is being burned by TGA from the surface of the silica gel.

The removal of the moisture content from the surface of the porous silica gel is observed from the actual TGA measurements (see Figure 3.7) by the appearance of a prominent peak in the derivative weight plot within the temperature range of 25 ^{0}C and 110 ^{0}C. Thus, the weight loss below 110 ^{0}C is attributed to the moisture content of the silica gel. The weight grafted is also computed using the formula that is clearly defined in section 2.3.2 of the previous chapter. This formula is as follows:

$$\textbf{S, (\%)} = \frac{(\text{Weight at } 110\ ^0\text{C} - \text{Weight at } 800\ ^0\text{C})}{\text{Weight at } 800\ ^0\text{C}} \times 100\%$$

When the heating temperature of the thermogravimetric analyzer reaches 800 ^{0}C, the grafted organic phase is completely burned leaving only the white solid particles of silica gel, which has a melting point[8] above 1000 ^{0}C. This is shown clearly in the TGA profile when the plot becomes constant starting at 700 ^{0}C, which simply means that no further compound is being burned from the sample.

The results of the TGA measurements for the cleavage of 3-(diethylenetriamino)propyl in THF/Methanol and water are shown in the Figures 3.8 and 4.9 respectively. The above calculation applies to the results. For the cleavage done in THF/methanol, the percent weight grafted due to the grafted organic phase dropped to 14% from 33.5%. Likewise, the percent weight of the cleavage done in water decreased to 7.5%. This proves the viability of the cleavage in solvents used, THF/methanol and in water.

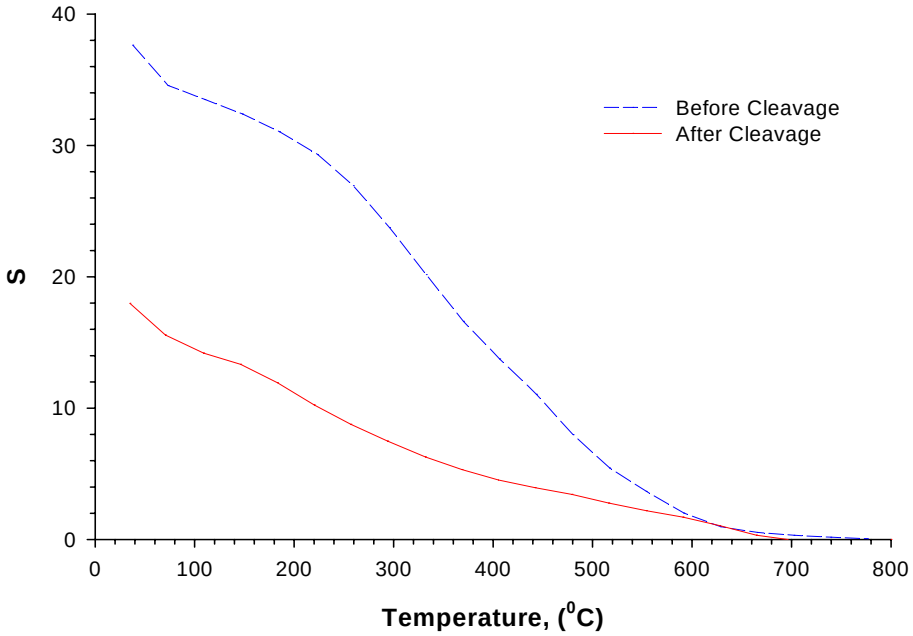


Figure 3.8 TGA profile of silica gel before and after cleavage of 3-(diethylenetriamino)propyl - functionalized silica gel in *THF/ methanol.*

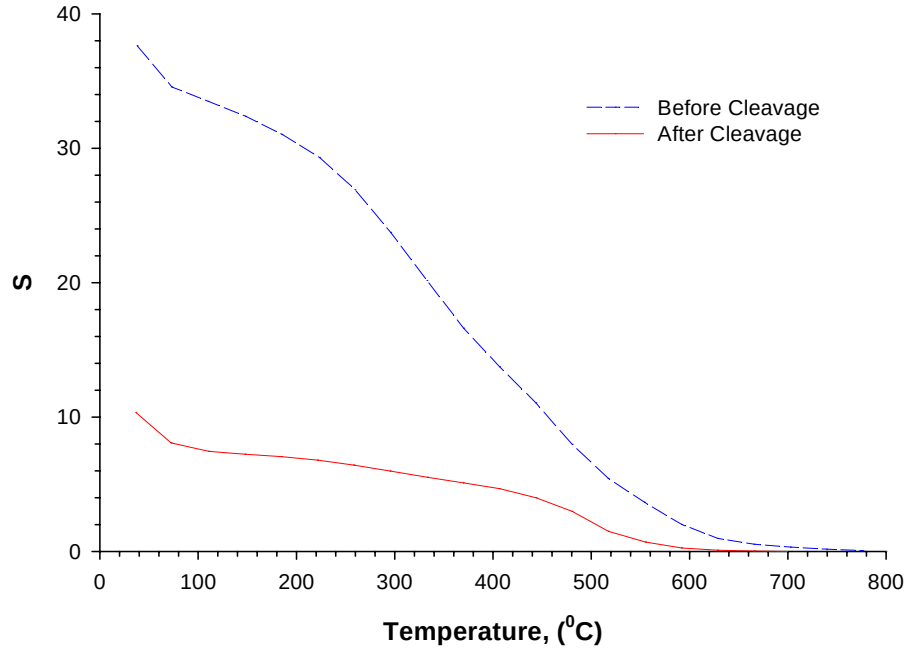


Figure 3.9 TGA profile of silica gel before and after cleavage of 3-(diethylenetriamino)propyl - functionalized silica gel in *water.*

To compare the three different methods, the efficiency of the cleavage was determined based on the TGA result and the calculation is as follows:

$$**\textbf{Cleavage Efficiency} = \frac{\text{Wt. loss before cleavage} - \text{Wt. loss after cleavage}}{\text{Wt. loss before cleavage}} \times 100\%$$

For more accurate results, the readings considered on the TGA profile were all taken at 110 ^{0}C. This is to eliminate the weight grafted due to moisture. The results of the calculation are summarized in Table 3.2.

Table 3.2 Summary of the cleavage efficiency of 3-(Diethylenetriamino)-propyl - functionalized silica gel based on TGA data.

Solvent Used	**Cleavage Efficiency (%*)
Tetrahydrofuran /Methanol	58.50
Methanol	82.68
Deionized Water	77.60

**percentage by weight*

Based on this computation, methanol proves to be the most effective solvent for the cleavage of the model compound. It has an efficiency of over 80%. As a result, the cleavage in methanol is favored for the cleavage of the bigger molecule, which is the PAMAM dendron grafted on silica gel.

3.3.3. Elemental Analysis

The elemental analyses were performed at the NUS Micro Analytical Laboratory with a Perkin Elmer 240C elemental analyzer for C, N, and F determinations.

Table 3.3 C and N analysis of silica gel before and after cleavage of 3-(diethylenetriamino)propyl.

Solvent Used	C (%*)		N (%*)		F (%)
	Before	After	Before	After	After
THF / Methanol	11.25	3.04	5.82	1.41	2.73
Methanol	11.25	1.07	5.82	0.92	0.38
Deionized Water	11.25	1.59	5.82	0.81	2.59

Table 3.4 Summary of the cleavage efficiency of 3-(Diethylenetriamino)-propyl - functionalized silica gel based on EA.

Sample	** Efficiency, (%*)	
	C	N
THF / Methanol	72.98	75.77
Methanol	90.49	84.19
Deionized Water	85.87	86.08

** percentage by weight*

*** Efficiency = (% before cleavage – % after cleavage) x 100%*
(% before cleavage)

The elemental analysis of the silica gel evidently shows the cleavage of the compound from the solid support. For instance, the percentage content of carbon and nitrogen atoms in the silica gel decreased after the cleavage reaction as presented in the Table 3.3.

The analysis shows that the silica gel contained fluorine atom despite of the alternate washing of the silica particles with methanol and water after the cleavage. The fluorine content of the silica gel after cleavage is summarized in

Table 3.3 for each of the solvents used during the reaction. This confirms the formation of the silicon-fluorine bond in the surface of silica gel after the cleavage of the organic phase (see mechanism at Scheme 3.2). The silicon-fluorine vibration was also identified by the infrared measurements. This vibration is clearly shown in the IR spectra of the silica gel after cleavage in three different solvents (see Figures 3.1, 3.2 and 3.3).

Finally, based on the elemental analyses results, the cleavage reaction done in methanol is the most efficient as compared to the other solvents like THF/methanol and water. The conformity of the results in the EA and TGA measurements strongly suggests that methanol is the most suitable solvent for the cleavage of 3-(diethylenetriamino)propyl from the silica gel. Thus, we preferred the use of methanol as the reaction solvent for the cleavage of the silica-grafted full generation PAMAM dendrons.

3.3.4. Mass Spectrometry

3.3.4.1 Tandem Mass (MS-MS) Spectrometry

A. Instrumentation. The instrument was calibrated before analysis of the sample. After calibration, the polymer sample was directly infused into the ESI source of Waters Quattro Micro API MS/MS spectrometer with a syringe pump flow of 5 μL/min flow rate. The capillary and the cone voltage were set at 3 kilovolts and 17 volts respectively. The MS/MS used argon as the collision-induced dissociation (CID) gas.

The sample was introduced into the ESI source in 50/50 (v/v) acetonitrile/water solution containing 50 millimolar ammonium acetate at concentrations approximately $1x10^4$ ppm. The acquisition range of the mass spectrum was from m/z 20 to 400, which was done in the positive ionization mode. The final spectrum was recorded and treated by using the MassLynx 4.0 software installed in the instrument.

B. Results and Discussions

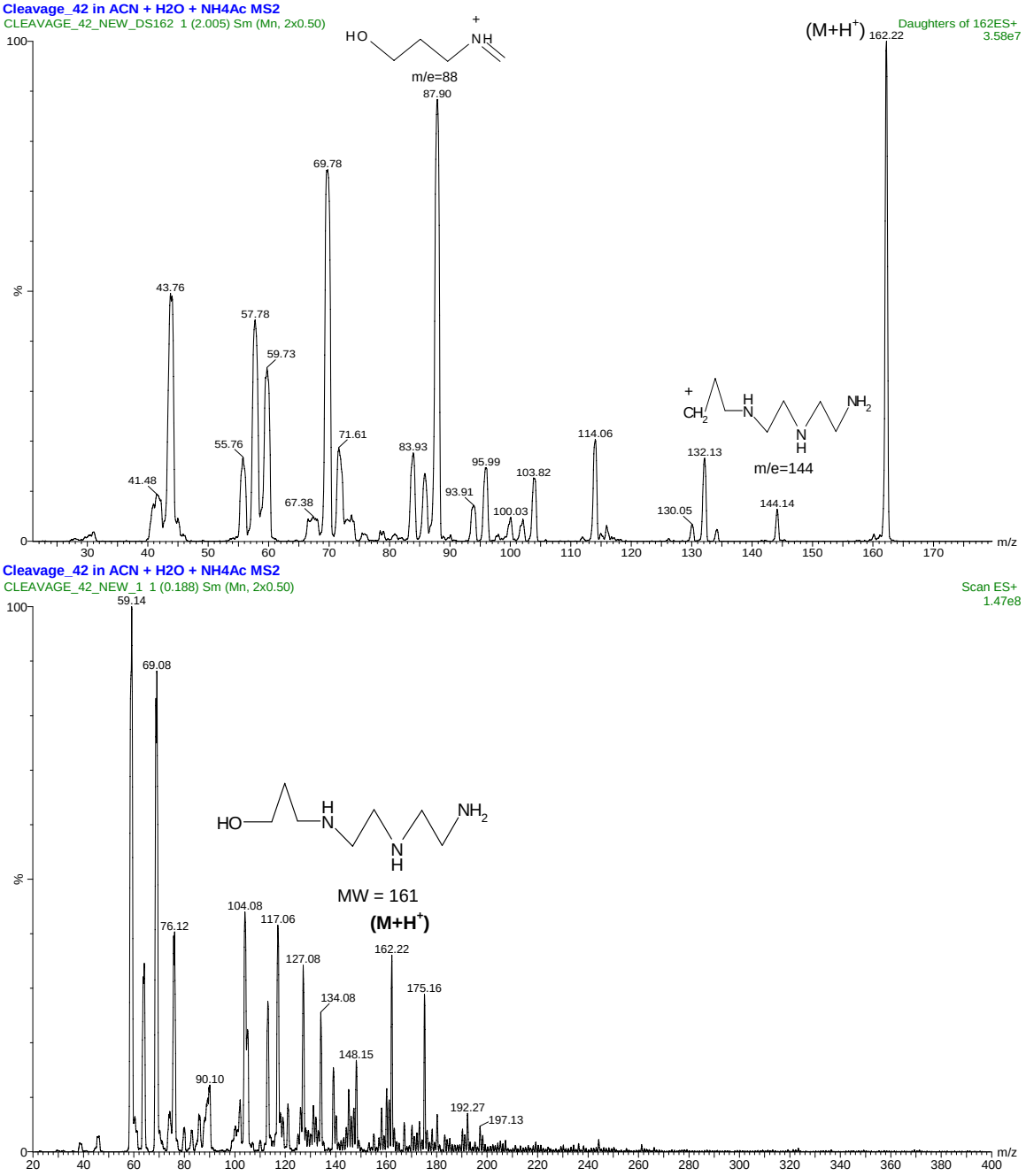


Figure 3.10 Tandem mass (MS/MS) spectrometry analysis of 3-(diethylenetriamino)propyl alcohol. Below is the MS parent scan of the compound while daughter scan is shown in the above spectrum.

The results of the mass analysis (see Figure 3.10) evidently showed that the sample is a mixture, which normally describes a polymer[9] because of the

distinct patterns of the peak present in the MS spectrum. Since the starting material is not pure that could be the reason why the purity of the 3-(diethylenetriamino)propyl - functionalized silica gel was not stated by the supplier. The end product after cleavage is expected to be a mixture of different components. Although purification of the cleavage sample was done using silica gel column chromatography, separation was difficult because the impurities contain the same polarity and functional groups as the target compound. This case is similar to that of the PAMAM dendron that was propagated on mesoporous spherical silica gel. During the solid phase synthesis of the macromolecule, several defects containing similar polarity and functional groups were also formed. As a result, the end product after the cleavage of the dendritic macromolecule from the solid support was a mixture of the target compound with the different structural defects, which were due to the propagation steps.

Although the end product was a mixture of several unknown components, the target compound could still be found undoubtedly in the sample. In the parent scan (lower spectrum in Figure 3.10), the target compound is obviously shown at m/z 162 ($MW+H^+$), which is the mass of the compound plus one additional unit. That can be accounted for a proton attaching the compound, making it charged because only charge species can be detected by the mass spectrometer. The other peaks observed in the parent scan are mainly due to the different components inside the mixture and are not due to fragmentation of the

compound because a soft ionization technique (ESI) was applied during the mass analysis.

To confirm the presence of the target compound in the sample, the peak at 162 was broken down using the CID gas. As a result, several fragments were found in the daughter scan (upper spectrum in Figure 3.10) that were not present originally in the parent scan. These fragments support the structure determination of the peak at m/z 162.

For instance after fragmentation, a distinguishing peak at m/z 88, which has the second highest intensity after 162, is shown only in the daughter scan. This illustrates that m/z 88 is a fragment of 162. This fragment is suggested to be a result of $CH_2NHCH_2CH_2NH_2$ removal in the structure of the target compound. $CH_2NHCH_2CH_2NH_2$ is neutral species that cannot be detected in the mass spectrometer. Another notable fragment is the peak at 144. This particular fragment is most probably a result of an OH removal at one terminal end of the compound. The above-mentioned fragments were also charged species; thus, their detection in the mass spectrometer is possible.

3.3.4.2 Time-of-Flight (TOF) Mass Spectrometry

A. Instrumentation. The Time-of-Flight mass analysis of the cleavage sample was done at JEOL Ltd., Japan using the new TOF MS instrument of model JMS-T100LC AccuTOF^{TM} equipped with MassCenter system version 1.3.1 software.

The orifice 1 and orifice 2 voltages were fixed at 40 and 3 volts respectively. And the acquisition range (m/z) was set from 100 to 600.

B. Results and Discussions

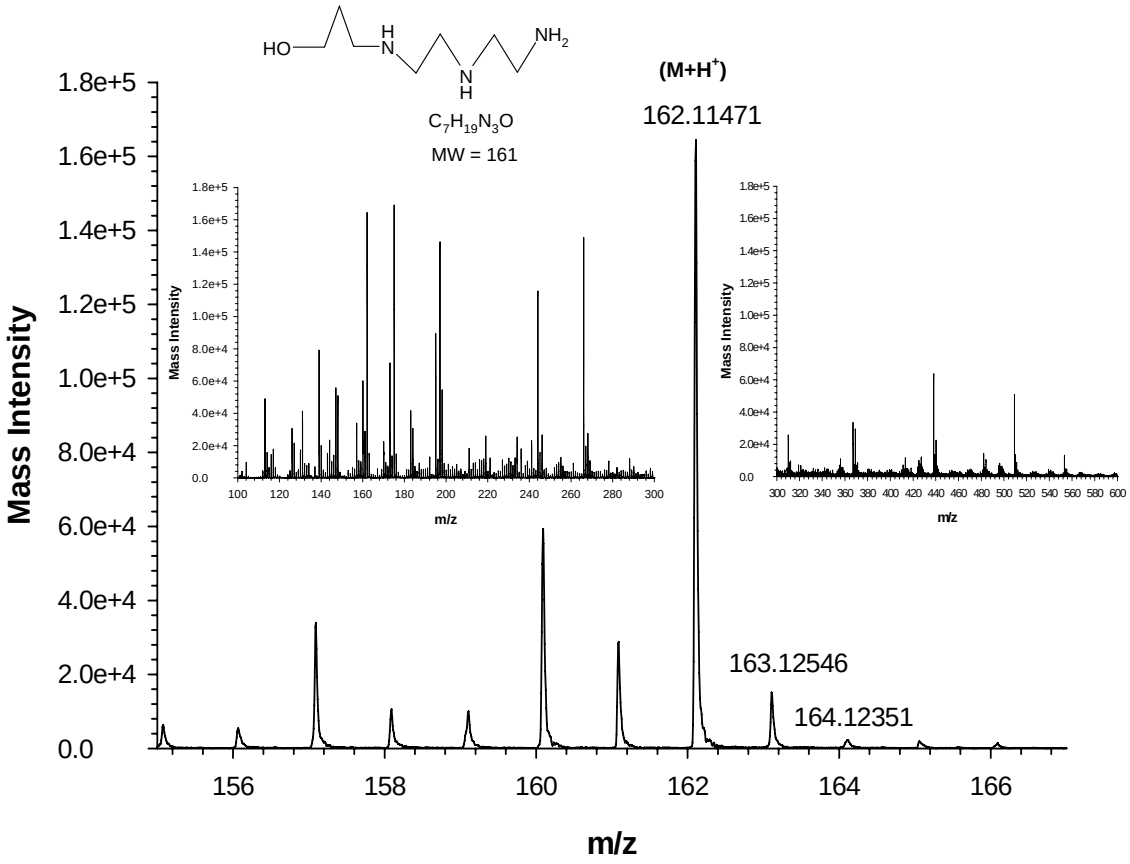


Figure 3.11 TOF mass spectrum of the 3-(diethylenetriamino)propyl alcohol. The full scan (m/z 100 to 600) is divided into two small diagrams above.

To confirm the result of the ESI MS-MS, the cleavage sample was sent for further analysis in the time-of-flight (TOF) mass spectrometry. The result of the analysis illustrates that the sample is undoubtedly a polymer[9] as shown by the distinct patterns of the peak in the spectrum (see the full scan on Figure 3.11). Interestingly, the peak at 162 that describes the target compound appeared also

in the TOF mass spectrum. Again, this peak is equivalent to the exact mass of the compound (161) with an addition of one unit due to the attached proton that made the compound charged.

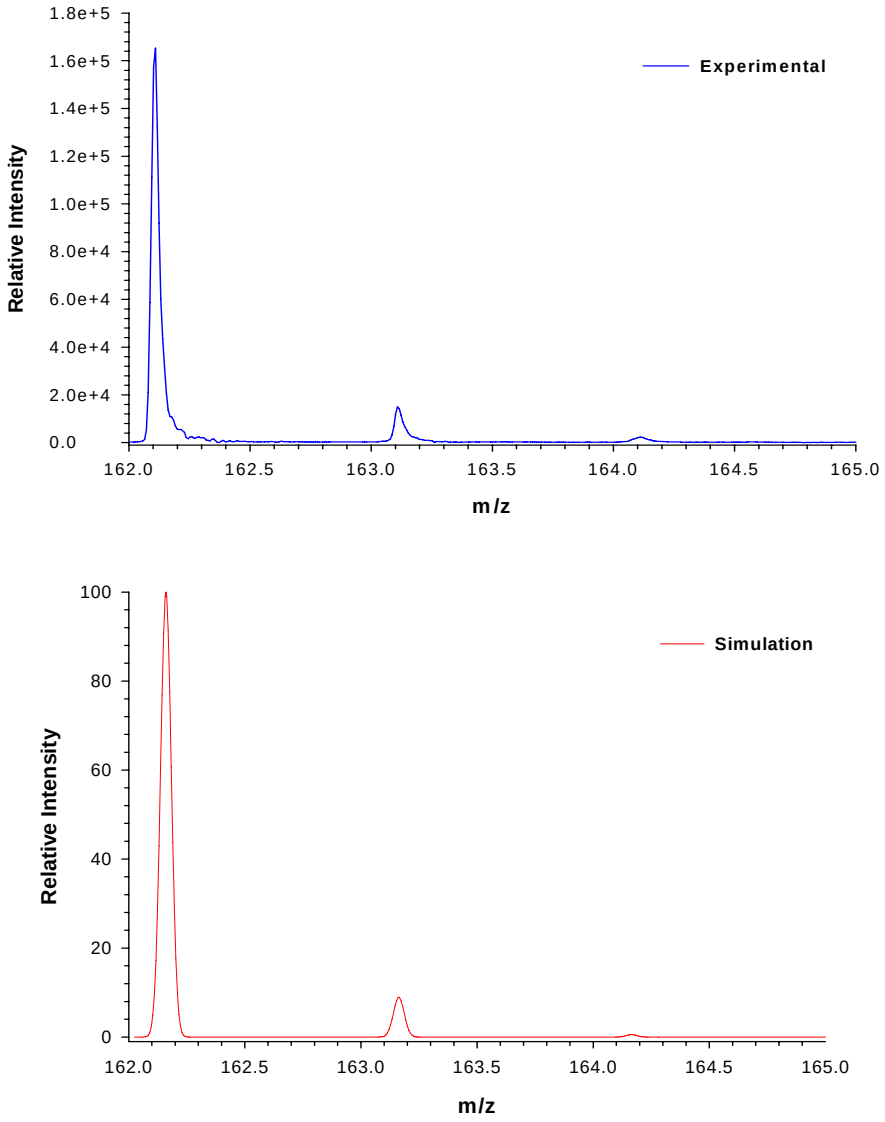


Figure 3.12 TOF mass analysis showing the isotopes of 162 (above); Simulation data of the peak at m/z 162 plus its isotopes (below).

Another evidence showing the presence of the target compound in the cleavage sample is the similar peak distribution profiles and the ratio of the isotopes in the experimental result and the simulation data taken from the given software and from the calculation in the molecular weight calculator. The experimental result and the simulation of the peak at m/z 162 are shown in the Figure 3.12.

3.4 Conclusion

It was shown that silica-grafted organic phase such as the 3-(diethylenetriamino)propyl can be successfully cleaved using hydrogen peroxide in the presence of potassium fluoride. Mass spectrometry gave the correct molar mass of the compound while infrared spectroscopy showed the important molecular vibrations of the different functional groups that are strong evidences of the presence of the compound in the cleavage sample.

Based on thermogravimetric and elemental analyses of the silica gel after cleavage of the 3-(diethylenetriamino)propyl, methanol illustrated the highest cleavage efficiency as compared to the other solvents used such as water and THF/methanol. Methanol is advantageous in the cleavage of dendritic macromolecules since they are most stable in this solvent according to literature.

3.5 References

1. Tamao, K.; Akita, H.; Iwahara, T.; Kanatani, R.; Yoshida, J.; Kumada, M. ***Tetrahedron***. 1983, 39, 983.
2. Fleming, I.; Henning, R.; Plant, H. ***J. Chem. Soc. Chem. Commun*****.** 1984, 29.
3. Stork, G.; Chan, T.Y.; Breault, G.A. ***J. Am. Chem. Soc.*** 1992, 114, 7578-7579.
4. Yang, A.; Li, T. ***Anal. Chem***. 1998, 70, 2827-2830.
5. Przybylski, M.; Glocker, M.O. ***Angew. Chem. Int. Ed. Engl.*** 1996, 35, 806-826.
6. Peterson, J.; Allikmaa, V.; Subbi, J.; Pehk, T.; Lopp, M. ***Eur. Polym. J.*** 2003, 39, 33-42.
7. Pavia, D.L.; Lampman, G.M.; Kriz, G.S. ***Intoduction to Spectroscopy***. 3rd edition.
8. http://www.azom.com/details.asp?ArticleID=1114.
9. Kallos, G.J.; Tomalia, D.A.; Hedstrand, D.M.; Lewis, S.; Zhou, J. ***Rapid Commun. Mass Sp.*** 1991, 5, 383-386.

Chapter 4: Cleavage of PAMAM dendrons from Porous Silica Gel

4.1 Introduction

There is presently no structural characterization of the PAMAM dendrons synthesized on solid support particularly silica gel. The silica-grafted PAMAM dendron can only be analyzed by few analytical techniques like infrared spectroscopy[1-4], thermogravimetry[2-4], inductively coupled plasma[2], and scanning electron microscopy[4]. Powerful analytical techniques like mass spectrometry and liquid-phase nuclear magnetic resonance (NMR) cannot be used to characterize the structure of the macromolecule because of the attached solid support. With the chemical cleavage of PAMAM dendron from the solid support, structural analysis can now be performed using mass spectrometry, which is considerably a more sensitive analytical tool than other conventional tools.[5] The analysis of the cleavage sample using liquid phase NMR is difficult to perform because the end product is a mixture of different components due to the solid phase synthesis of the macromolecule. Cleavage of PAMAM dendrons from the silica gel followed by analysis using mass spectrometry has not been realized previously.

With the success of the new improved method in the cleavage of the commercially available compound 3-(diethylenetriamino)propyl shown in the previous chapter, the same condition was applied to cleave the dendritic macromolecule from the silica gel, except that the reaction was done at room temperature for longer times. And the reaction was done only in methanol

because it gives the highest efficiency among the other solvents tested during the cleavage of the commercial compound. Further, methanol is essential for the reaction because the macromolecule is said to be stable[10] in methanol. That is why the commercial PAMAM dendrimer supplied by Sigma- Aldrich is dissolved only in methanol.

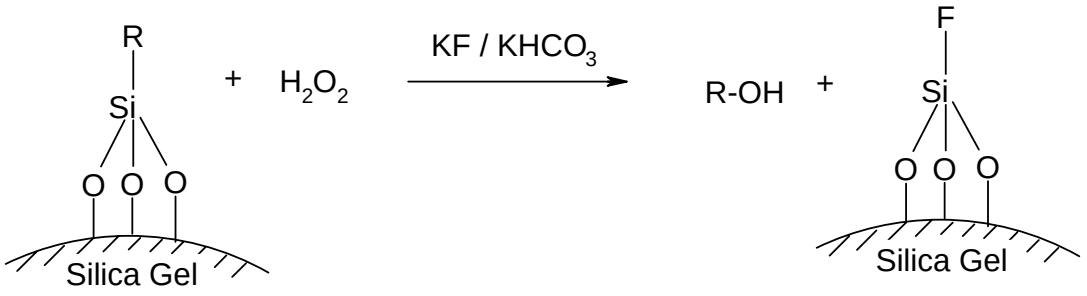
After cleavage, the PAMAM dendron, already in liquid form, was analyzed by mass spectrometry using chemical ionization mode. By applying this technique to analyze the dendritic macromolecule, some useful information about the structures of the PAMAM dendron including the synthesis “failures” is only determined convincingly.

The use of this powerful analytical technique like mass spectrometry has been increasingly rapid recently due to the advent of methods that are capable of producing gaseous ions from the macromolecules.[6] The earliest groups who applied chemical ionization mass spectrometry to analyze the PAMAM dendrimers synthesized in liquid phase was Tomalia[7] and co-workers. Further, G.J. Kallos and co-workers[8] also did successful studies on the analysis of the liquid-phase synthesized G4 PAMAM (Starburst) dendrimer using mass spectrometry.

Infrared spectroscopy was also used to check the important molecular vibrations of the different functional groups present in the cleavage dendritic

macromolecule. Furthermore, the result of the IR spectra of the self-synthesized PAMAM dendron was compared to that of the commercially synthesized PAMAM dendrimer on the same generation that had exactly the same functional groups.

After cleavage, the silica gel was analyzed by thermogravimetry (TGA), elemental analysis (EA), and infrared spectroscopy. The efficiency of the method was computed from the TGA and EA measurements using the data before and after the cleavage of the macromolecule. The theoretical illustration of the cleavage of PAMAM dendron from the silica gel is depicted in Scheme 4.1. The same mechanism applies for the cleavage of the dendritic macromolecule (refer to Scheme 3.2). The ideal structures of G1 and G2 PAMAM dendrons are shown in Figure 4.1.



R = (G1, G2) PAMAM Dendron

Scheme 4.1 Theoretical illustration of the cleavage of PAMAM dendrons.

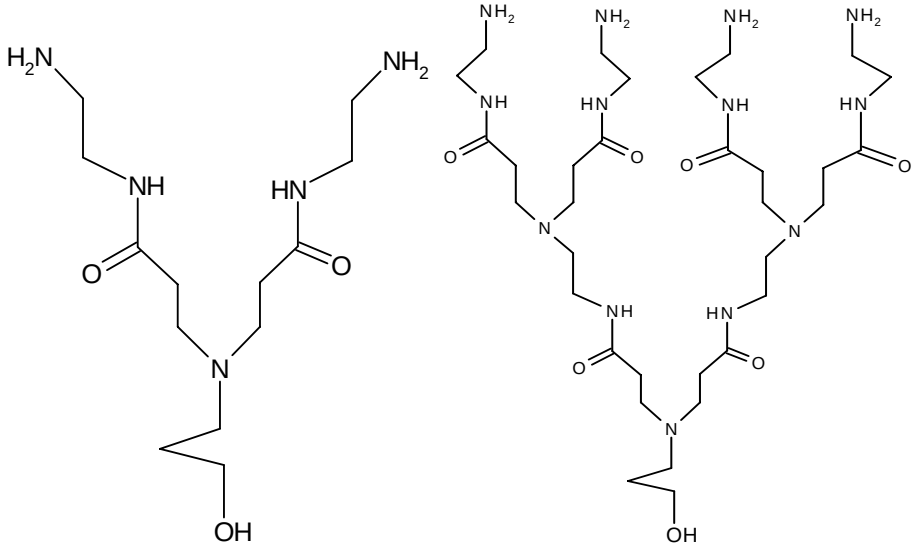


Figure 4.1 Theoretical structure of G1 PAMAM dendron (MW 303) and *(left)* G2 PAMAM dendron (MW 759).

4.2 Experimental

4.2.1 Materials

The solid-phase synthesized PAMAM dendrons were kept in a dry cabinet at constant temperature of 20 ^{0}C while the 30% aqueous hydrogen peroxide supplied by Kanto Chemical Co., Inc. was stored in the fridge at low temperature. The solvents used were purchased from Sigma-Aldrich, J.T. Baker, or Merck. Potassium bicarbonate from Sigma-Aldrich and potassium fluoride supplied by Riedel-de Hgen were also kept in the dry cabinet to avoid moisture absorbance from the surrounding atmosphere. Filter paper, Whatman 42 (retention size, 2.5 microns) purchased from United Scientific Equipment Pte Ltd was used in the vacuum filtration.

4.2.2 Procedures

The cleavage reaction of PAMAM dendron from the surface of silica gel was done in methanol using hydrogen peroxide (H_2O_2) in the presence of potassium fluoride (KF) and potassium bicarbonate ($KHCO_3$). This was carried out as follows: Into a 100 ml flask that contained 1 gram of solid (silica-grafted PAMAM dendron) dissolved in methanol (30 ml), potassium fluoride (10 equivalents to the organic phase) and potassium bicarbonate (10 equiv.) were added to the mixture. Afterwards, 30% aqueous hydrogen peroxide (10 equiv.) was added into the batch reactor using the micropipette, and then the mixture was stirred constantly for 48 hours using a magnetic stirrer with a speed of 400

rpm. The reaction conditions of the cleavage reaction are summarized in Table 4.1.

Table 4.1 Summary of the cleavage reaction conditions.

Reaction Time	48 hours
Temperature	Room temperature
Reagents	4.) 30% aq. H_2O_2 - 10 equiv. to the organic phase 5.) Potassium Carbonate - 10 equiv. to the organic phase 6.) Potassium Flouride - 10 equiv. to the organic phase
Solvent	30 milliliters of Methanol per 1 gram of solid (silica-grafted-PAMAM dendon)

After the reaction, the solvent was removed under vacuum using the rotary evaporator. The residue was dissolved in a small amount of solution containing 10% methanol in dichloromethane and was transferred into the glass column packed with silica gel for purification. After washing with 10% methanol in dichloromethane (total volume about 300 milliliters) using the column, the filtrate was concentrated to yield the desired compound, which was analyzed by infrared spectroscopy and mass spectrometry.

The silica gel inside the column was retrieved using the pipette filler. It was then washed with the methanol followed by large amounts of de-ionized water to remove the inorganic components adsorbed onto the surface of the silica gel. After washing, the solid support was dried in vacuo at 110 ^{0}C for 8 hours to remove the trace amount of reaction solvent left and moisture present on the surface of the silica gel. Finally, the silica gel was analyzed by infrared spectroscopy, thermogravimetry, and elemental analysis.

4.3 Characterizations and Interpretation of Results

4.3.1. Infrared Spectroscopy

A. Methodology

c.) **Silica Gel**. Before the IR measurement, the solid sample was dried in tube oven (at 110 ^{0}C, for 8 hours), which was connected to a vacuum with liquid nitrogen contained in a Dewar flask as interphase to trap the organic solvent and moisture that were removed from the sample by the vacuum pump.

The analysis of silica gel was done at 40 scans having 4 cm^{-1} resolutions using the Digilab Excalibur FTIR. Initially, potassium bromide was pressed into a translucent disc using a hydraulic press and was run into the instrument as the background. Subsequently, the sample was prepared by mixing the silica gel with potassium bromide and was pressed into a translucent disc also under vacuum suction. The spectra were quantified in terms of absorbance units.

d.) **Cleavage compound**. A translucent disc of potassium bromide was made first using the hydraulic press; and then the cleavage compound, a pale yellow viscous liquid, was pasted onto the center of disc. The sample was run into the instrument at 40 scans and 4 cm^{-1} resolutions. Potassium bromide was also scanned as the background before the sample measurement. The spectra were quantified in terms of absorbance units.

e.) **PAMAM Dendrimer**. Initially, the methanol was vaporized using a vacuum at 40 ^{0}C to concentrate the compound, which was dissolved in methanol as prepared commercially by the supplier. After evaporating the solvent, the compound was blow-dried using nitrogen gas for at least 5 minutes to remove completely the trace amount of solvent left in the sample.

Afterwards, translucent disc of potassium bromide was again made using the hydraulic press; and then the PAMAM dendrimer, also a pale yellow viscous liquid, was pasted onto the center of disc. Finally, the sample was run into the instrument at 40 scans and 4 cm^{-1} resolutions. The spectra were also quantified in terms of absorbance units.

B. Results and Discussions

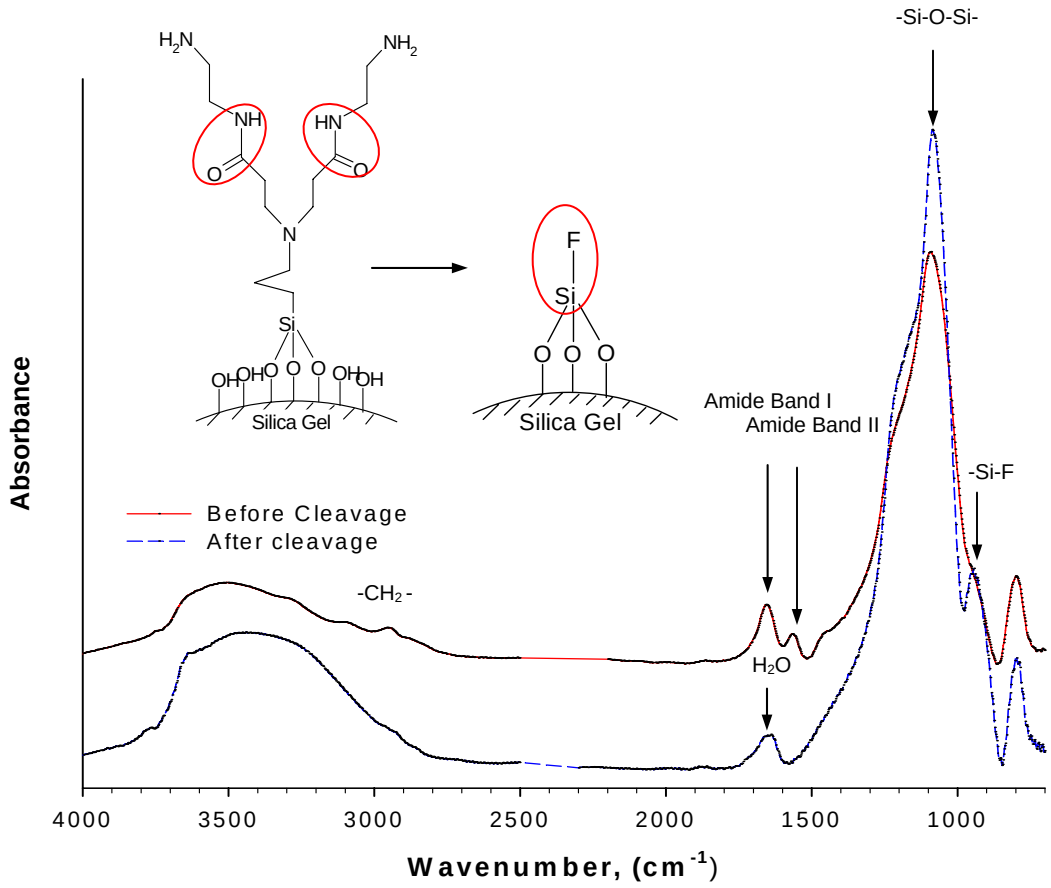


Figure 4.2 IR spectrum of silica gel before and after cleavage of *G1* PAMAM dendron.

The cleavage of G1 PAMAM dendron from the solid support is vividly observed in the IR spectra (see Figure 4.2) by the disappearance of the amideband I at 1655 cm^{-1} and amide band II at 1550 cm^{-1}. [9] This shows that a full generation PAMAM dendron has amide functional groups. After the cleavage of the compound, the two amide peaks are replaced by another peak at 1750 cm^{-1}, which is due to moisture[9]. Similarly, the C-H bond stretching[9] of the CH_2 functional groups of the compound found in the series of small peaks near 3000 cm^{-1} disappear, showing the cleavage of the organic phase from the silica gel.

Another evidence in the spectra is the appearance of the stretching vibration of the silicon-fluorine bond[9] at 950 cm^{-1} after the cleavage reaction. This peak is not visible in the IR spectrum of the silica-grafted PAMAM dendron.

After the cleavage, the two-amide[9] peaks (1655 and 1550 cm^{-1}) that disappeared in the IR spectrum (refer to Figure 4.2) of the silica-grafted G1 PAMAM dendron are now seen in the spectrum of G1 PAMAM dendron (see Figure 4.3). This showed the cleavage of the compound from the solid support.

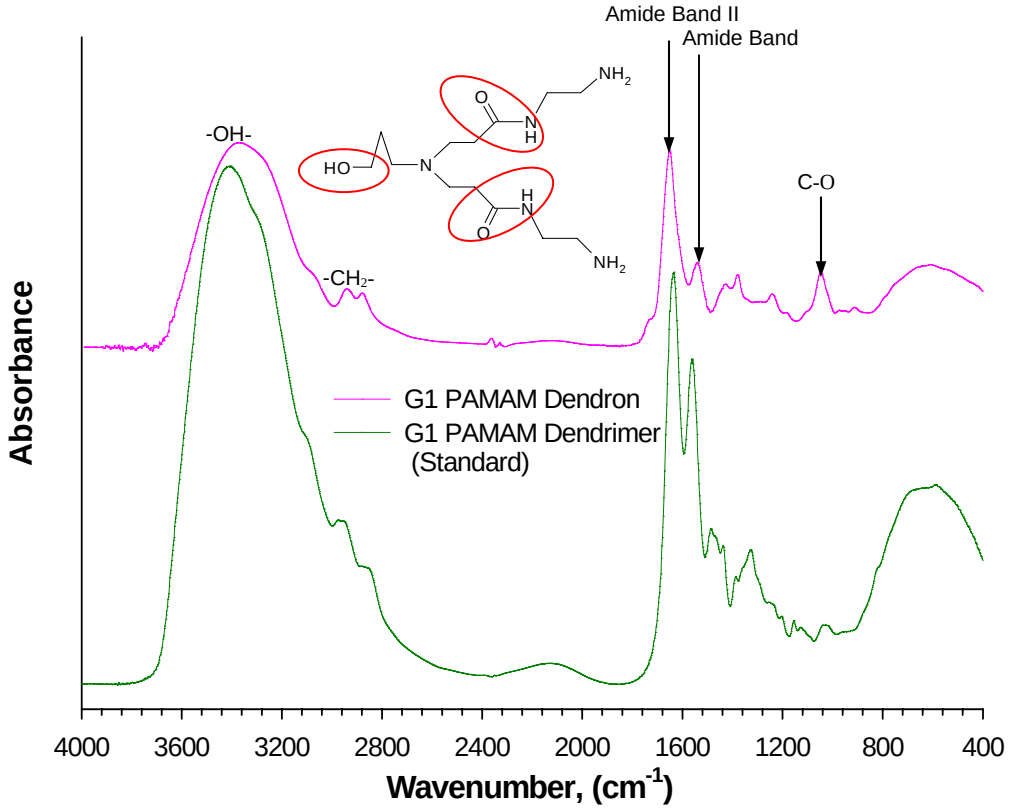


Figure 4.3 Liquid phase IR spectrum of *G1* PAMAM dendron compared to the reference spectrum of *G1* PAMAM dendrimer.

The amide band I assigned to the –C=O vibrations[10] and amide band II attributed to the N-H bending vibrations in plane coupled with the valency C-N vibrations[10] are shown in two prominent peaks in the IR spectrum of the compound that is in liquid phase. Then, comparing it with the spectrum of the PAMAM dendrimer having the same generation, the two amide peaks present in G1 PAMAM dendron are located exactly on the region (wavenumber) as the two peaks found in the PAMAM dendrimer, which can only be assigned to amide band I and amide band II. The ideal structure of G1 PAMAM dendrimer is depicted in Figure 4.4. Further, a C-O single-bond stretching vibration[9] is observed at 1050 cm^{-1}. This IR band is found only in the spectrum of G1 PAMAM dendron because the structure of PAMAM dendrimer does not have this functional group. This confirms that the final product has OH functionality.

Finally, the CH_2 vibrations[9] indicated by the series of small peaks near 3000 cm^{-1} are still present in the IR spectrum of the compound. With these evidences, G1 PAMAM dendron was successfully cleaved from the silica gel. The dendritic macromolecule is now present in liquid phase similar to PAMAM dendrimer that is purchased commercially.

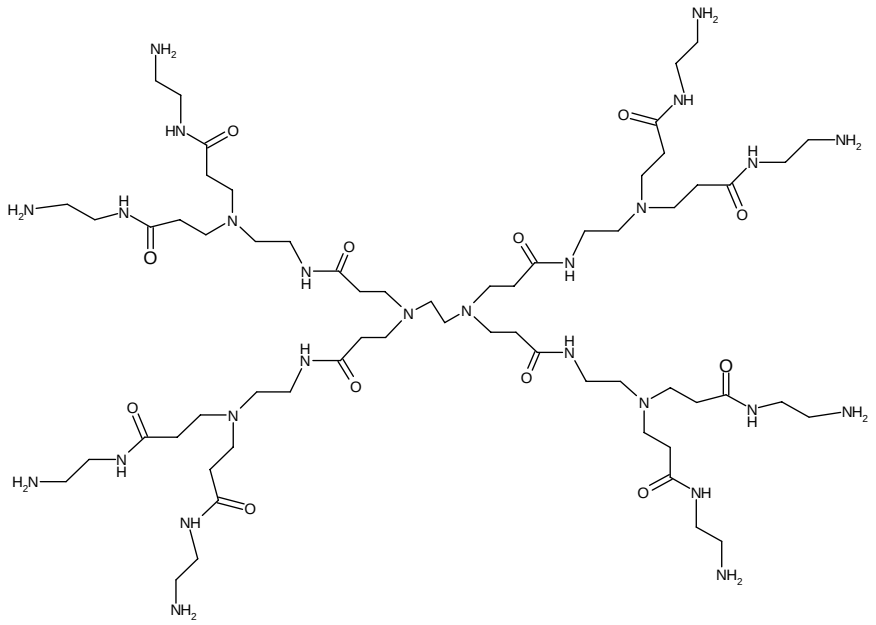


Figure 4.4 Theoretical illustration of EDA core *G1* PAMAM dendrimer.

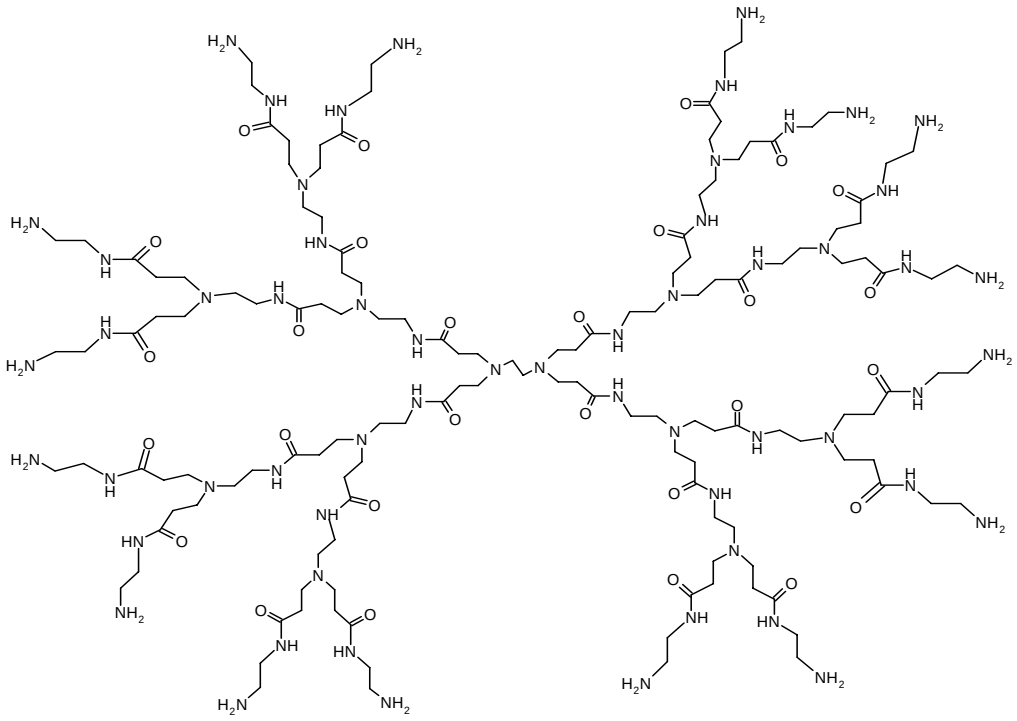


Figure 4.5 Theoretical illustration of EDA core *G2* PAMAM dendrimer**.**

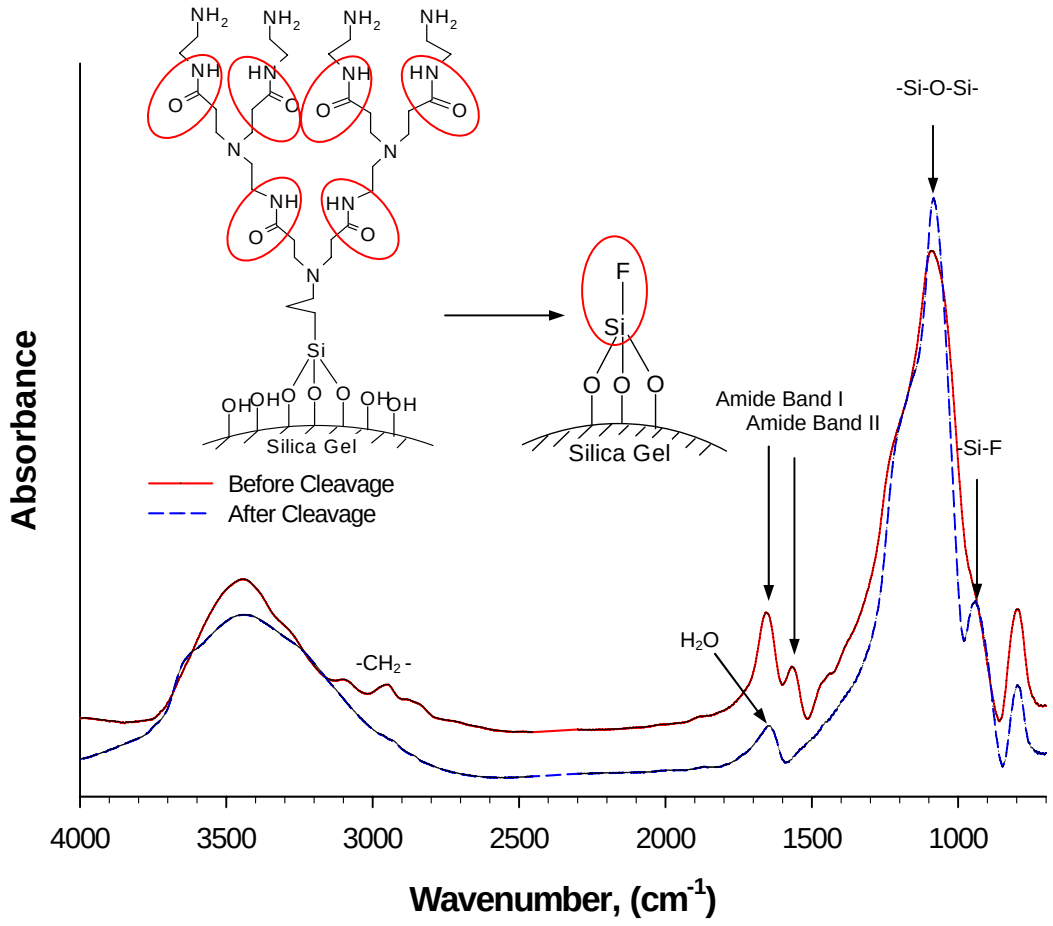


Figure 4.6 IR Spectrum of silica gel before and after cleavage of *G2* PAMAM dendron.

The cleavage of G2 PAMAM dendron from silica gel was also successful as shown in the IR spectrum (see Figure 4.6) by the disappearance of the amide band I[9] and amide band II at 1655 and 1550 cm^{-1} respectively[9]. After the cleavage, the amide peaks were replaced by a moisture peak at 1750 cm^{-1}, assigned to OH stretching vibrations[9]. The CH_2 vibrations[9] observed in the series of small peaks near 3000 cm^{-1} also disappeared, and the stretching vibration of the silicon-fluorine bond[9] at 950 cm^{-1} appeared in the IR spectrum of the silica gel after the compound was cleaved.

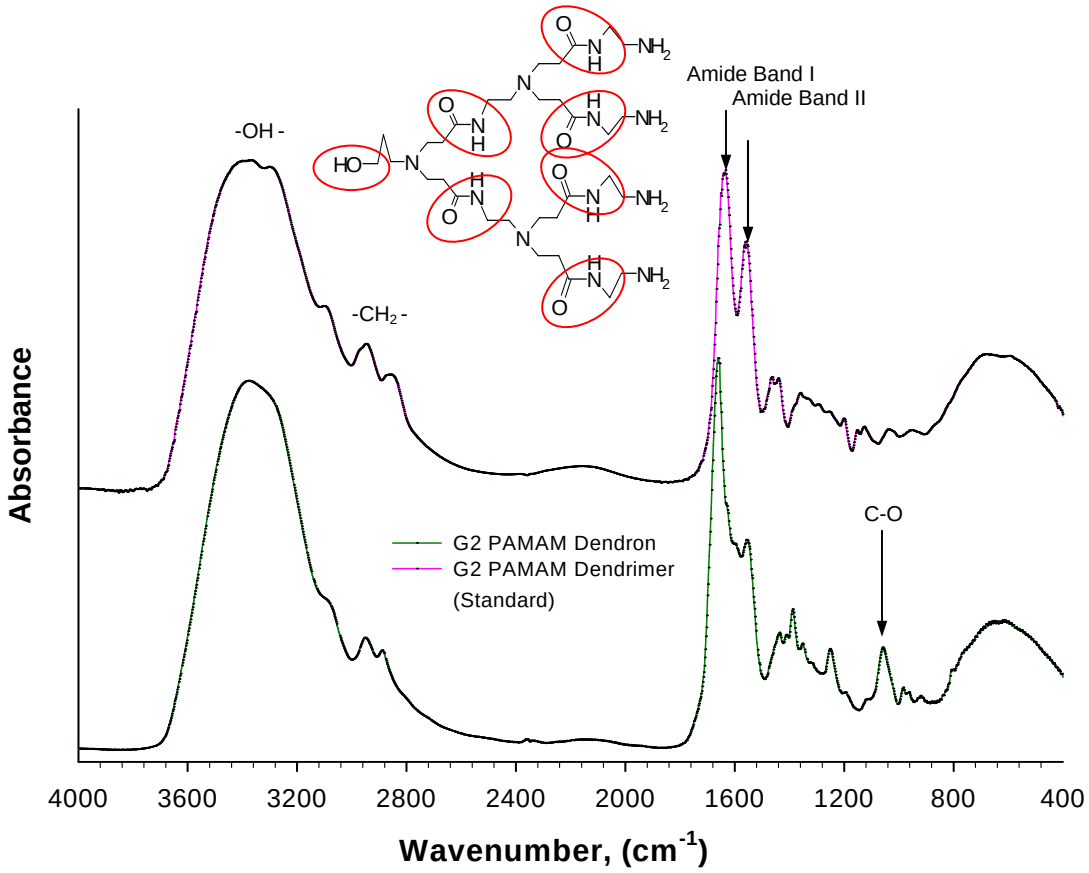


Figure 4.7 Liquid phase IR spectrum of *G2* PAMAM dendron compared to the reference spectrum of *G2* PAMAM dendrimer.

The spectrum of G2 PAMAM dendron in liquid phase (refer to Figure 4.7) contains the same amide peaks that are present in the IR spectrum of the silica gel grafted with G2 PAMAM dendron. These peaks were also present in the IR spectrum of G2 PAMAM dendrimer whose ideal structure is found in figure 4.5. The series of small peaks near 3000 cm^{-1} corresponding to C-H bond stretching[9] of the CH_2 functional groups are also found in the IR spectrum. Further, the C-O single bond stretching vibration[9] at 1050 cm^{-1} is found only in the spectrum of the

dendron. This proves that G2 PAMAM dendron is now in liquid phase after its cleavage from the solid support.

4.3.2. Thermogravimetry

A. Methodology

Before the TGA analysis, the solid sample was dried in tube oven (at 110 ^{0}C, for 8 hours), which was connected to a vacuum with liquid nitrogen contained in a Dewar flask as interphase to trap the organic solvent and moisture that were removed from the sample by the vacuum pump.

To compare accurately the result of the TGA measurements before and after cleavage, the same method was used in the instrument to analyze the samples. About 15 milligrams of the solid sample was loaded into the platinum TGA crucible. Initially, the TGA was set to isothermal setting for 5 minutes before heating to purge the unwanted gases from the sample compartment. Using air with a flow rate of 200 milliliters per minute, the sample was heated from room temperature to 800 ^{0}C with constant temperature ramp of 5 ^{0}C per minute. When the final temperature was reached by the system, the instrument cooled down automatically using the same gas that was used for heating the sample.

Thermogravimetric analyzer supplied by TA instruments of model SDT 2960 was used to analyze the silica gel. The instrument is equipped with the Universal Analysis software to view the TGA curve of the sample after burning.

B. Results and Discussions

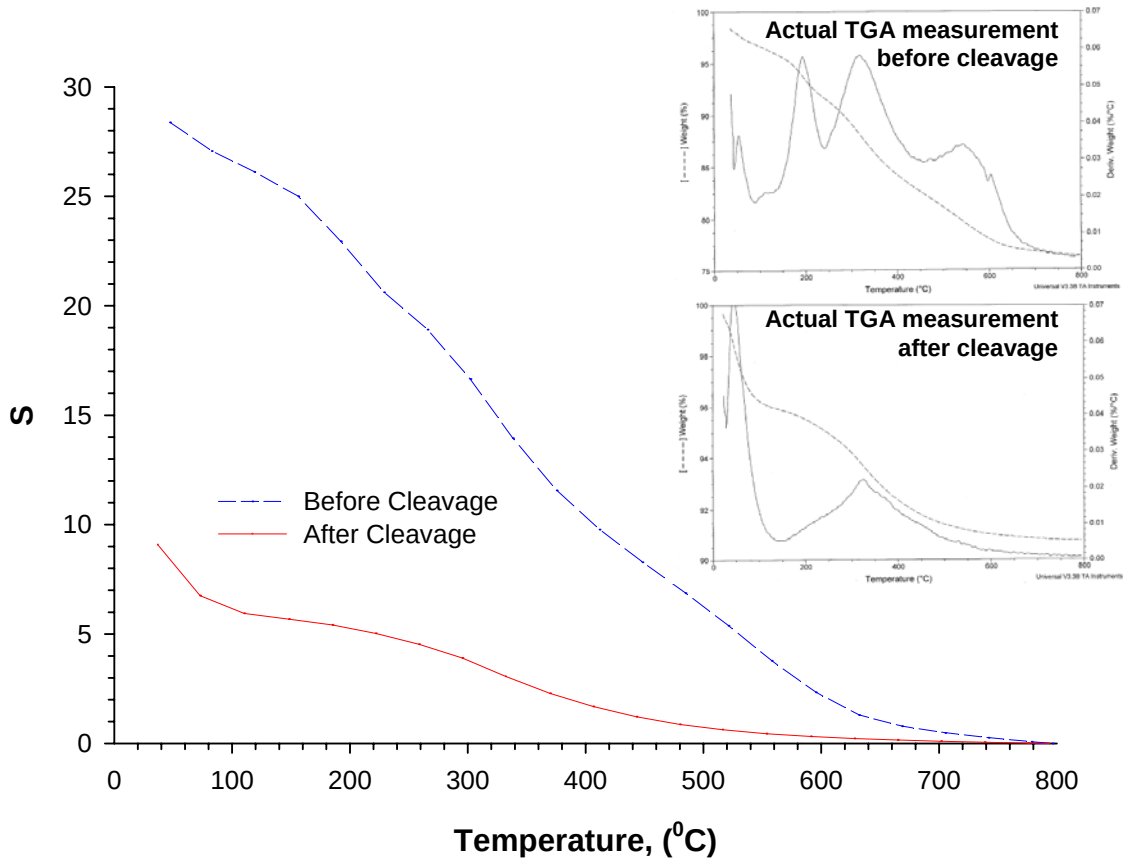


Figure 4.8 TGA profile of silica gel before and after cleavage of *G1* PAMAM dendron.

The outcome of the TGA measurement supports the result of the IR analysis showing the cleavage of the compound from the solid support. To illustrate, the percent weight grafted of the organic phase in the surface of silica gel dropped to 5.95%, which was originally 26.4% before the cleavage of the G1 PAMAM dendron (see Figure 4.8). Similarly after the cleavage of G2 PAMAM dendron, the percent weight grafted of the organic phase decreased from 35.4% to 7.9% as shown in Figure 4.9. Again, the readings from the TGA profile were all taken at 110 ^{0}C to disregard the weight of the moisture content of the porous silica gel, which is below 110 ^{0}C.

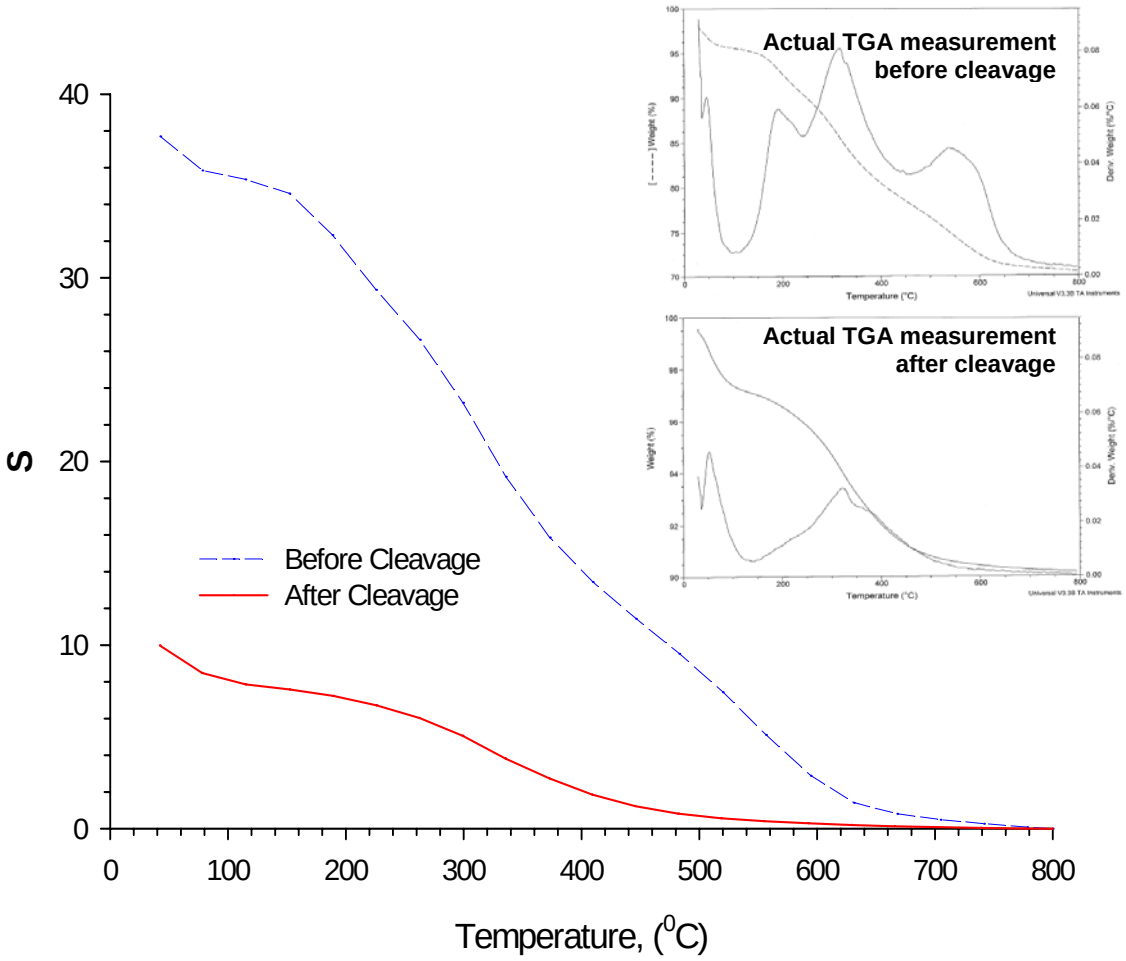


Figure 4.9 TGA profile of silica gel before and after cleavage of *G2* PAMAM dendron.

The percent weight grafted was calculated based on the formula:

$$\textbf{Weight grafted, \% (S)} = \frac{(\text{Weight at } 110\ ^{0}\text{C} - \text{Weight at } 800\ ^{0}\text{C})}{\text{Weight at } 800\ ^{0}\text{C}} \times 100\%$$

To determine the effectiveness of the dendritic macromolecule cleavage, the efficiency was computed based on the TGA measurements of G1 and G2 PAMAM dendrons. In order to achieve accurate results, the readings from the TGA profile were all taken at 110 ^{0}C to disregard the percent weight grafted due to moisture in the calculation. To compute for the cleavage efficiency, the percent

weight grafted of the bare silica gel was considered. It was noted in the experiment that the bare silica showed a 4% weight grafted after burning in the TGA instrument.

Under the current conditions of the new improved cleavage method, the efficiency of the dendritic macromolecule cleavage is about 90%. This is much higher than the result of the cleavage of the model compound**,** 3-(diethylenetriamino)propyl - functionalized silica gel that has an efficiency of only 83% (refer to table 4.2). Hence, the cleavage of PAMAM dendrons from silica gel using hydrogen peroxide in methanol gives a very high efficiency on the TGA measurement. The results of the calculation are summarized in Table 4.2.

Table 4.2 Summary of the Cleavage Efficiency of PAMAM Dendrons Based on TGA Data.

Sample	**Cleavage Efficiency (%)
G1 PAMAM Dendron	92.53
G2 PAMAM Dendron	88.42

**percentage by weight*

$$\textbf{\textit{Cleavage Efficiency}} = \frac{\textit{Wt. Loss Before Cleavage – Wt. Loss After Cleavage}}{\textit{Wt. Loss Before Cleavage-Wt. Loss of Bare Silica}} \times 100\%$$

4.3.3. Elemental Analysis

The elemental analysis was also performed in NUS Micro Analytical laboratory with a Perkin Elmer 240C elemental analyzer for C, N and F determinations.

Table 4.3 C and N analysis of silica gel before and after cleavage of PAMAM dendrons.

Sample	C (%*)		N (%*)		F (%)
	Before	After	Before	After	After
Cleavage in Methanol					
G1 PAMAM Dendron	11.19	1.73	3.73	<0.50	0.42
G2 PAMAM Dendron	13.42	2.1	4.77	<0.50	0.52

Table 4.4 Summary of the cleavage efficiency of PAMAM dendrons based on EA.

Sample	**Efficiency, (%*)	
	C	N
Cleavage in Methanol		
G1 PAMAM Dendron	84.5	>86.6
G2 PAMAM Dendron	84.3	>89.5

** percentage by weight*

*** Efficiency = (% before cleavage – % after cleavage) x 100%*
(% before cleavage)

The C and N elemental analyses of the silica gel clearly show the cleavage of G1 and G2 PAMAM dendrons. For instance, the weight percent of both atoms (see Table 4.3) in the surface of silica gel decreased after the cleavage reaction. The efficiency was computed based on the given formula**. The results of the computation are given in Table 4.4. Based on EA measurements, the efficiency is over 80% if the carbon atom is taken into consideration and about 90% if the nitrogen atom is considered.

The fluorine atom was also detected in the silica gel by elemental analysis despite thorough washing of the silica particles with methanol and water after the cleavage reaction. This shows the formation of a strong silicon-fluorine bond at the surface of the silica gel replacing the silicon-carbon bond. The fluorine content of the silica gel is listed in Table 4.3. Previously, the stretching vibration of the silicon-fluorine bond at the surface of the silica gel was determined by the infrared spectroscopy (refer to Figures 4.3 and 4.6).

In table 4.3, the percentage of fluorine in G2 PAMAM dendron is higher than the percentage content of G1 PAMAM dendron. This is so because G1 and G2 PAMAM dendrons were synthesized in a different batch. It just happened that during the synthesis of G2 PAMAM dendron more initiator sites were immobilized (see Table 4.5). This is possible because the silylation step that is the immobilization of the initiator sites into the silica gel is difficult to control and non-uniform as mentioned earlier in chapter 2.

Table 4.5. Grafted initiator site for G1 and G2 PAMAM dendrons

PAMAM Dendron	Weight of Initiator Site Grafted
Generation 1	10.5801
Generation 2	12.8810

Elemental analysis shows similar result with the TGA measurements. Therefore, the current method can cleave the dendritic macromolecule from the silica gel at high efficiency.

4.3.4. Mass Spectrometry

4.3.4.1. Instrumentation

The analysis was performed using Finnigan Mat 95XP. This mass spectrometer that uses chemical ionization technique has a capability of analyzing a sample in both low resolution to get the nominal mass and high resolution to measure the accurate mass of the compound. For singly charged ions below m/z 1000, nominal mass is adequate and convenient.[11]

The sample was introduced into the instrument by direct insertion probe method. The mass spectrometer was operated in positive chemical ionization mode with methane gas as reagent gas. The source conditions used for the instruments were as follows: temperature 138 ^{0}C, electron energy 120 eV, accelerating voltage 5kV and ionization current 0.2 mA.

4.3.4.2. Results and Discussions

A. G1 PAMAM Dendron

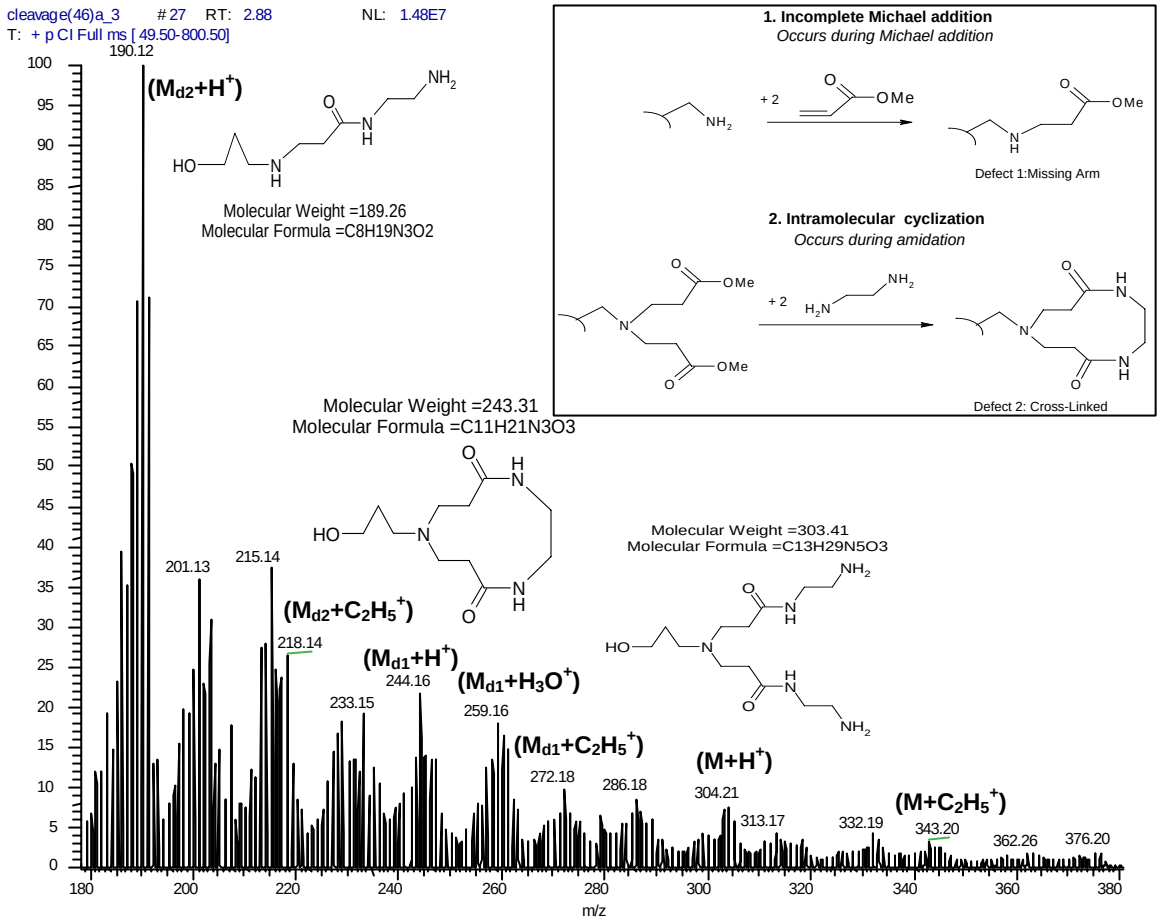


Figure 4.10 Mass spectrum of *G1* PAMAM dendron after cleavage from silica gel at *room temperature* for *48 hours.*

The end product after the cleavage of G1 PAMAM dendron is a mixture of the ideal compound plus probable defects (see Figure 4.11) that occur most likely at this generation during the solid phase synthesis of the macromolecule. This is clearly shown in the mass spectrum on Figure 4.10. The result demonstrates that the sample is a polymer whose mass analysis is similar to that of the PAMAM dendrimer. [8]

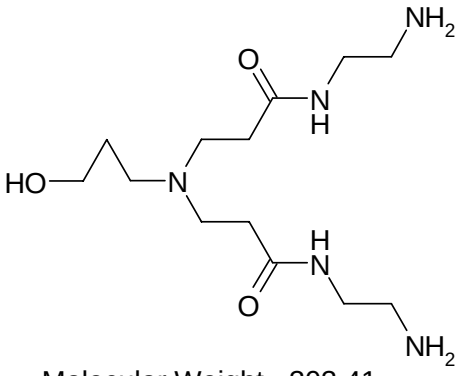
Although the reaction was done at mild conditions without using heat and purification was performed using the column after the reaction, the result of the cleavage is still a mixture of several components. This is possible because the solid phase synthesis produces several defects during the silica gel propagation of PAMAM dendron. This is the reason for failure to achieve the theoretical amount of grafting during the experiment (see Table 2.2 and Figure 2.8). This is also similar to the case of the other research groups[1-4,6,8,11] who propagated PAMAM dendrons on solid support like silica gel. The two side reactions that produce the defects during the synthesis are the incomplete propagation in the Michael addition step and the cross-linking during the amidation reaction. Furthermore, separation of the organic components in the cleavage sample using the column is difficult because the defects contain similar polarity and functional groups as the ideal structure. The column was effective only in removing the inorganic components that were used in the reaction to cleave the macromolecule from the silica gel.

The peaks at m/z 304 and 343 show the ideal compound, which is an addition of H^+ and $C_2H_5^+$ respectively. Further, the peaks at m/z 190 ($189+H^+$) and 218 ($189+C_2H_5^+$) belong to the same defect (MW 189) that is due to incomplete Michael addition during the propagation, causing unsymmetrical structure (defect 2 on Figure 4.11).

Another defect was found in the mass spectrum as shown by the three peaks at m/z 244, 260 and 272. This defect (defect 1 on Figure 4.11) is a result of a cross-linking reaction that occurs in the amidation step of the solid phase synthesis. M/z 244 is an addition of H^+ into the mass of the cross-link (MW 243), while m/z 260 is an addition of H_3O^+. Also, m/z 272 belongs to the cross-link defect that is a result of adding $C_2H_5^+$ into its mass. These defects can be easily ionized by the mass spectrometer as compared to defect 3 (see Figure 4.11) because of the presence of the terminal amino groups in their structure that can easily accept a proton in the outer shell.[12] Thus, only the defects 1 and 2 were determined by the mass analysis. It is also possible that defect 3 was not formed during the propagation of G1 PAMAM dendron, therefore it cannot be detected in the mass analysis.

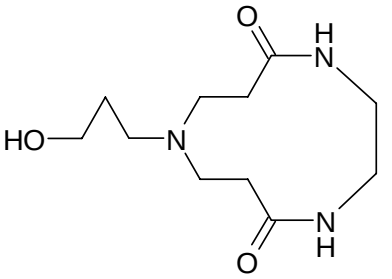
Generation 1 PAMAM Dendron

Ideal Strcuture



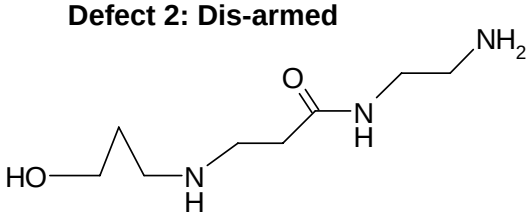
Molecular Weight =303.41
Molecular Formula =$C_{13}H_{29}N_5O_3$

Defect 1: Crosslink



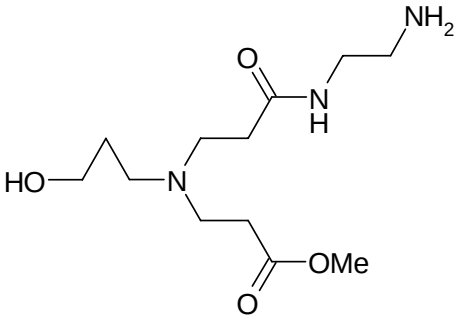
Molecular Weight =243.31
Molecular Formula =$C_{11}H_{21}N_3O_3$

Defect 2: Dis-armed



Molecular Weight =189.26
Molecular Formula =$C_8H_{19}N_3O_2$

Defect 3: Dis-armed



Molecular Weight =275.35
Molecular Formula =$C_{12}H_{25}N_3O_4$

Figure 4.11 Ideal structure of G1 PAMAM dendron and the suggested structures of the faulty synthesis products that are most likely to occur at this generation.

The silica-grafted G1 PAMAM dendron was also cleaved at 60 ^{0}C for 12 hours, and the purification was also done using the column. At this condition, the peak of the ideal compound (m/z 304) is also seen in the spectrum (Figure 4.12) with an addition of H^+ into its mass. Moreover, the cross-linked defect is obviously seen at m/z 244 that is also an addition of H^+.

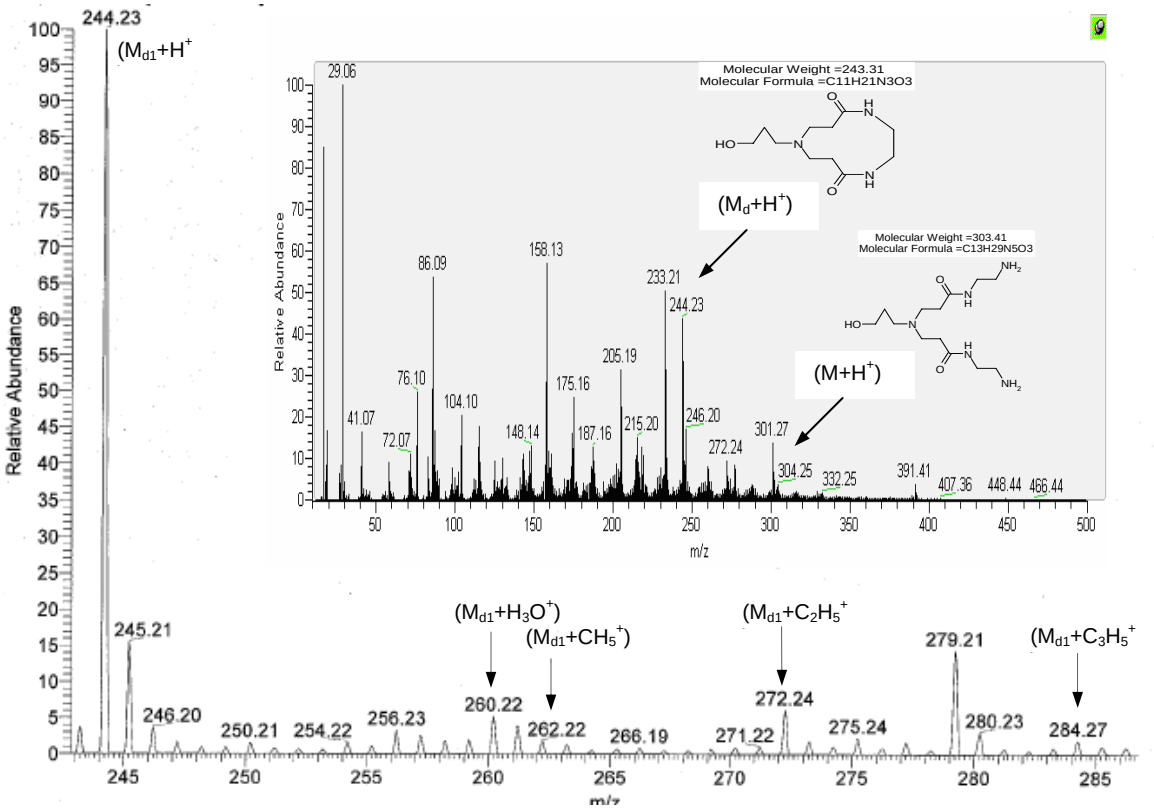


Figure 4.12 Mass spectrum of *G1* PAMAM dendron after cleavage from silica gel at *60 ^{0}C* for *12 hours*. The full scan of this spectrum is also shown in this figure with a range of m/z 0 to 500.

If we expand the mass spectrum starting at m/z 243 up to 285, several peaks can clearly describe the cross-linked defect (see Figure 4.12). This is due to the different plasma ions that can possibly attach into the compound during the analysis. These are H^+ (addition of 1 unit into the mass of the cross-linked), CH_5^+ (addition of 19 units), $C_2H_5^+$ (addition of 29 units), and $C_3H_5^+$ (addition of 41 units). The peaks of the plasma ions are clearly shown in the full scan of the mass spectrum on Figure 4.12. With these evidences, the cross-linked defect undoubtedly occurs in the cleavage sample of G1 PAMAM dendron.

B. G2 PAMAM dendron

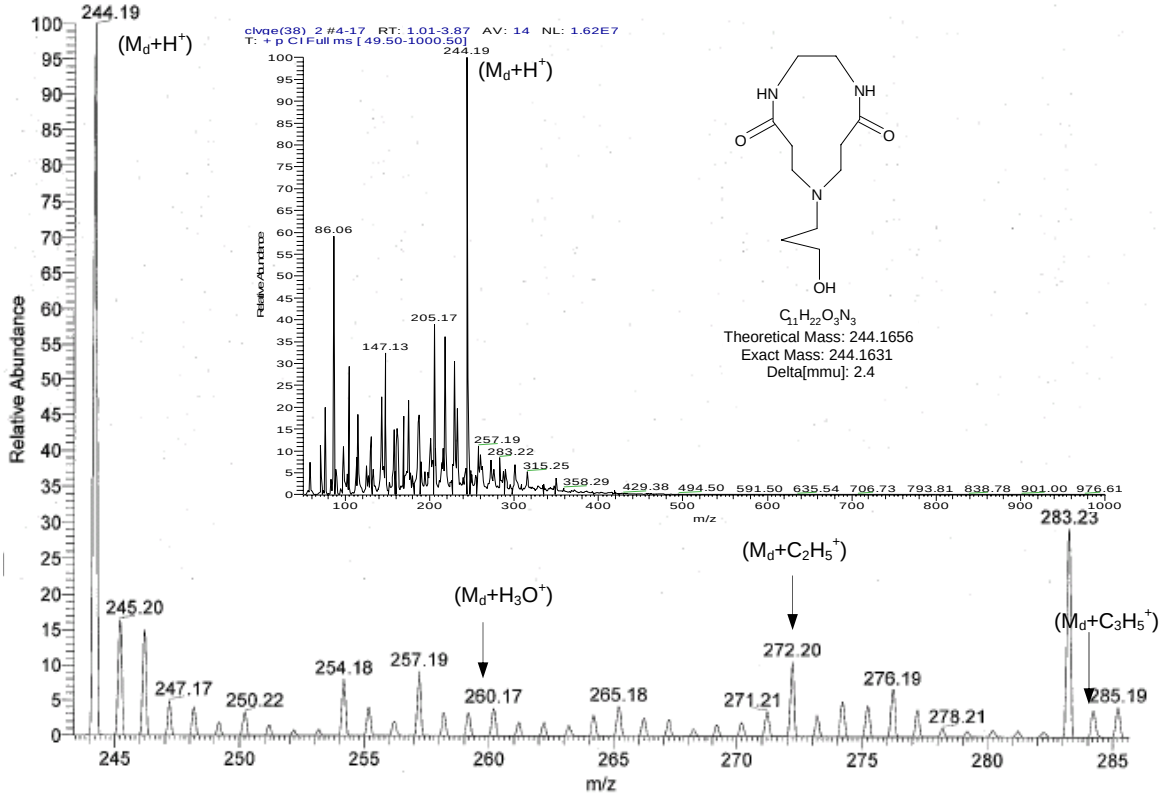


Figure 4.13 Mass spectrum of G2 PAMAM dendron after cleavage from the silica gel at *60 ^{0}C* for *12 hours*. The full scan of the spectrum of G2 is also shown in a range from m/z 0 to 1000.

The mass analysis of the cleavage sample containing G2 PAMAM dendron contains the peak at m/z 244, which is the mass assigned to the cross-linked defect. This peak, which is an addition of H^+ into the mass of the cross-linked, is very prominent as shown in the full scan of the mass spectrum on figure 4.13. The mass of the ideal compound (MW 759) having no structural defect is no longer visible in the spectrum because the relative abundance of the target compound is very low unless the spectrum is expanded many times, which we find meaningless.

The different plasma ions that can possibly be attached into the compound during the analysis produce several peaks that reveal the presence of cross-linked defect in the cleavage sample of G2 PAMAM dendron. The peaks at m/z 244, 260, 272 and 284 belong to the same compound, which is the cross-linked defect. These peaks are shown in the Figure 4.13.

For singly charged ions below m/z 1000 like the cleavage PAMAM dendrons, nominal mass is adequate and convenient.[13] Since the nominal mass abundance of the peak at m/z 244 is relatively high as compared to the other peaks present in the spectrum, the instrument can accurately determine the exact mass of that particular signal. The result of the exact mass measurement shows that the theoretical mass is close to the exact mass of the cross-linked defect, and the error (2.4 mmu) is within the acceptable range.

Although the mass of the target compound cannot be found in the spectrum of G2 PAMAM dendron, the exact mass of the cross-linked defect is accurately measured. Only a few ideal structure of G2 PAMAM dendron is synthesized during the silica gel propagation of the macromolecule because more defects[14] are formed in the higher generations as compared to G1 PAMAM dendron. Thus, the mass spectrometer cannot give a strong signal in spectrum for the target compound having an ideal structure. Previous analysis by TGA (Table 2.2) and FTIR (Figure 2.2) show that more defects are formed in the G2 PAMAM dendron.

4.4 Conclusion

The method used to cleave the commercially available silica-grafted small polymer is also successful in the cleavage of the self-synthesized full generation PAMAM dendrons. Both infrared spectroscopy and mass spectrometry strongly verified the presence of the macromolecule in the sample. Furthermore, structural defects that are mainly due to the stepwise synthesis of the PAMAM dendrons were determined by mass analysis of the cleavage sample. This finding strongly supports that cross-linking and incomplete Michael addition reactions happen during the silica gel propagation of the PAMAM dendron. As a result, the theoretical amount of propagation was not achieved during the experiment (see Table 2.2).

4.5 References

1. Tsubukawa, N.; Ichioka, H.; Satoh,H.; Hayashi, S.; Fujiki, K. ***React. Funct. Polym.*** 1998, 37, 75-82.
2. Bu, J.; Judeh, Z.M.A.; Ching, C. B., Kawi, S. ***Catal. Lett.*** 2003, 85(3-4), 183-187.
3. Li, R.; Bu, J. ***Korean J. Chem. Eng.*** 2004, 21(1), 98-103.
4. Bu, J.; Li, R.; Quah, C.W.; Carpenter, K.J. ***Macromolecules***. 2004, 37, 6687-6694.
5. Egner, B.; Langley, J.; Bradley, M. ***J. Org. Chem.*** 1995, 60, 2652-2653.
6. He, M.; McLuckey, S.A. ***Rapid Commun. Mass Sp.*** 2004, 18, 960-972.
7. Tomalia, D.A.; Baker, H.; Dewald, J.; Hall, M.; Kallos, G.; Martin, S.; Roeck, J.; Ryder, Smith, P.; ***Polym. J.*** 1985, 17(1), 117-132.
8. Kallos, G.J.; Tomalia, D.A.; Hedstrand, D.M.; Lewis, S.; Zhou, J. ***Rapid Commun. Mass Sp.*** 1991, 5, 383-386.
9. Pavia, D.L.; Lampman, G.M.; Kriz, G.S. ***Intoduction to Spectroscopy***. 3rd edition.
10. Nikolic, L.; Skala, D.; Nikolic, V.; Stamenkovic, J.; Dragan, B.; Ilic-Stojanovic, S. ***J. Appl. Polym. Sci.*** 2004, 91, 387-395.
11. Wang, C.; Zhu, G.; Li, J.; Cai, X.; Wei, Y.; Zhang, D.; Qiu, S. ***Chem. Eur. J.*** 2005, 11, 4975-4982.
12. Peterson, J.; Allikmaa, V.; Subbi, J.; Pehk, T.; Lopp, M. ***Euro. Polym. J.*** 2003, 39, 33-42.
13. van der Wal, Sj.; Mengerink, Y.; Brackman, J.C.; de Brabander, E.M.M.; Jeronimus-Stratingh, C.M.; Bruins, A.P. ***J. Chromatogr.*** 1998, 825, 135-147.
14. Peterson, J.; Ebber, A.; Allikmaa, V.; Lopp, M. ***Proc. Estonian Acad. Sci. Chem.*** 2001, 50, 3, 156-166.

.

Chapter 5: Conclusion and Suggestions for Future Work

5.1 Conclusion

Silica gel propagation of PAMAM dendrons using alternate Michael addition and amidation reactions was successful. However, formation of structural defects during propagation was also inevitable. This contributed to the low amount of grafting, and made purification difficult after cleavage. Consequently the final product was a mixture of the macromolecule and its defects, which is normally the case of a polymer sample.

Defects were formed as products of the side reactions that occurred during the two iterative steps. These reactions are incomplete Michael addition that are brought about by steric hindrance and cross-linking of the adjacent terminal amine groups. Side reactions are more extensive in the propagation of the higher generations; thus, the efficiency of grafting decreases as generation increases. Between the two side reactions aforementioned, cross-linking mostly contributed to the low amount of grafting as determined by thermo-gravimetric analysis. With the peaks of the defects also found in the mass spectrum of the cleavage sample, side reactions really occur during the propagation of PAMAM dendrons.

Hydrogen peroxide can be used to cleave an organic phase like PAMAM dendrons from the silica gel support at its carbon-silicon bond. Furthermore,

cleavage of the silica-grafted amine terminated commercial compound are feasible in three different solvents namely, (50% v/v) THF/methanol, methanol, and water. Nevertheless, methanol gives the highest efficiency in both thermo-gravimetric and elemental analyses. Using methanol as solvent for the cleavage reaction of PAMAM dendrons gives an advantage because dendritic macromolecules are most stable in methanol. Unexpectedly, cleavage of the full generation PAMAM dendrons showed an efficiency of about 90%, which is higher than the cleavage efficiency of the smaller polymer.

With wide applications of the silica-supported PAMAM dendrons, improvement of the solid-phase synthesis still remains a great challenge. Finding other suitable cleavage methods is also necessary because there are numerous applications of the macromolecules in the liquid phase. Moreover, cleavage of PAMAM dendrons followed by mass spectrometric analysis is a very good approach that can prove that the macromolecule is successfully synthesized onto the silica gel support.

5.2 Suggestions for Future Work

Because of the limited time we have only achieved this far but we still believed that much can be done on this field of research with the rapid developments of the solid phase synthesis in the last decade and the numerous interesting applications of dendritic macromolecules in both solid and liquid phases. Furthermore, this is the very first attempt on the cleavage of PAMAM

dendrons from the silica gel. In the succeeding paragraphs are some valuable recommendations that can be carried out for further studies.

The cross-linking phenomenon in the amidation step is most likely to occur because of the flexibility in the design of the structure of the synthesized dendron. This problem contributed mostly to the low efficiency of grafting. Thus, introduction of other functional groups that would make the structure of the dendron rigid can be considered to avoid or lessen the problem of cross-linking during propagation. This might improve the efficiency of the synthesis and reduce the possibility of forming defects that is making the purification of the compound difficult after the cleavage reaction.

Further study on the application of the silica-grafted PAMAM dendrons can be done. For instance, formation of a particular catalyst through complexation with metal is one useful application. Functionalization of the full generation PAMAM dendrons after the solid phase synthesis is another work that can also be considered before cleaving the dendritic macromolecule from the support. For instance, functionalizations of free amine terminal groups can lead to the production of functional PAMAM dendrimers with various properties that are useful to some applications like anti-microbial agents[1], flame retardancy[2], etc.

The current research on chemical cleavage of the silica-grafted macromolecules can be extended to the half-generation ester-terminated

PAMAM dendrons. The cleavage of these dendrons can be carried out under the same conditions, except that it has to be done at a much lower temperature like 0^0C to minimize the occurrence of saponification of the ester functional groups. Since the decrease in temperature slows down the kinetics of the cleavage reaction, the reaction time can also be prolonged to achieve higher efficiency. A study of temperature effects on the cleavage reaction can be done and analysis of the product using mass spectrometry is also essential.

Finding other purification techniques to isolate the ideal compound from the defects or to reduce the number of components within the product can be done. Purification of the product after the cleavage is one big challenge in this research work. The use of a suitable membrane to separate the compound from the side products is one consideration. Membrane separation is convenient and effective. Furthermore, polyacrylamide gel electrophoresis can be applied for the separation of PAMAM dendrons to evaluate the purity of the macromolecules. Electrophoresis is simple, rapid and inexpensive separation technique. Previously, Sharma[3] and co-workers developed a simple polyacrylamide gel electrophoresis procedure for the separation of polyamidoamine dendrimers.

Propagation of PAMAM dendrons onto the surface of non-porous silica gel can also be exploited. This reduces the synthesis cost of the macromolecules since non-porous material is much cheaper than its porous counterparts. The non-porous material needs only to be treated prior to the immobilization of the

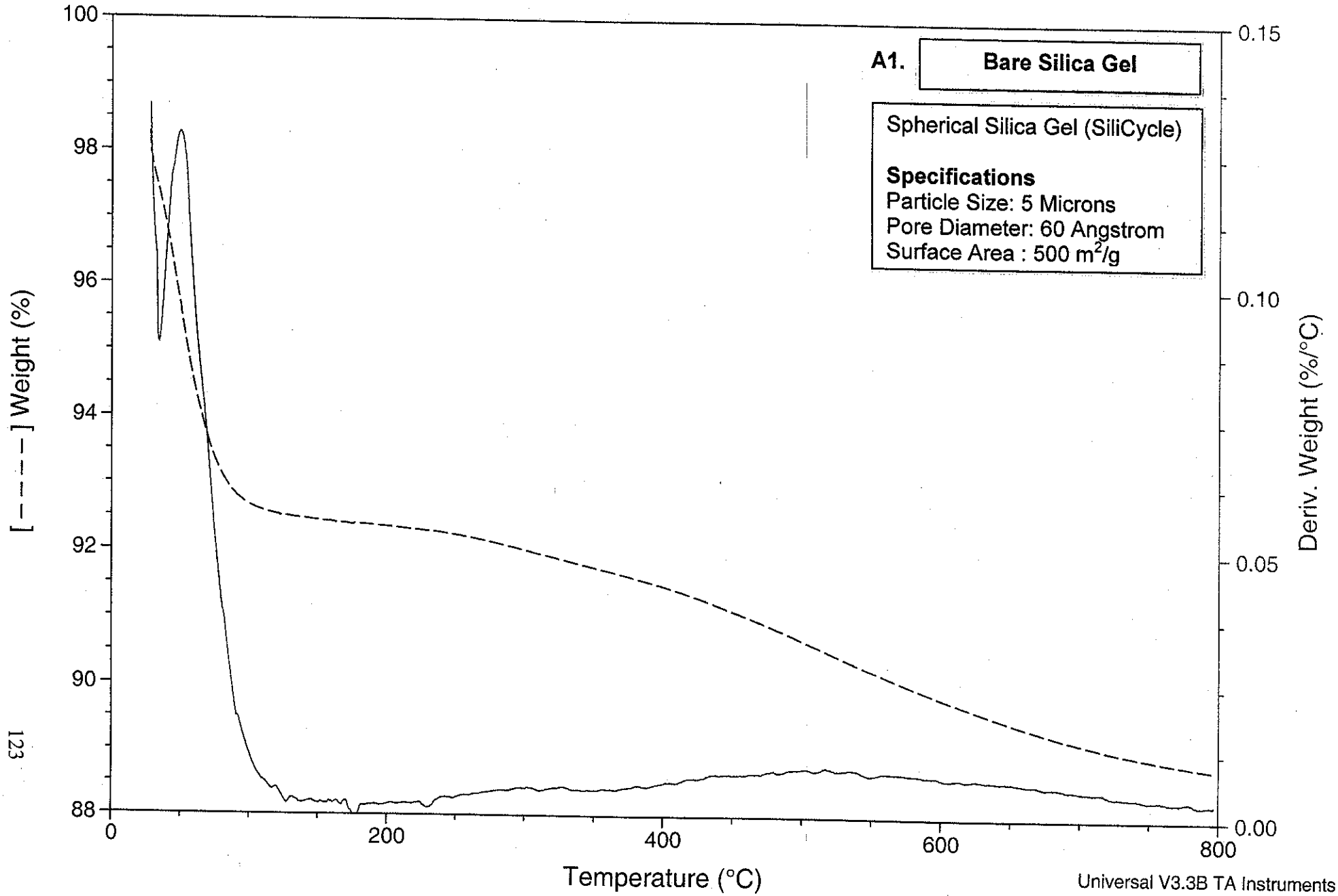
initiator site to activate its surface. One way of activating the surface is by acid treatment that is simple and non-costly. Cohen[4] and co-workers did previous work regarding the grafting of vinyl acetate onto the non-porous silica gel.

5.3 References

1. ***US Patent 6579906:*** Dendrimer Biocide-Silver Nanocomposites: their preparations and applications as potent anti-microbials.
2. Gillingham, E.L.; Lewis, D.M.; Srikulkit, K.; ***Color. Technol***. 2001, 117, 6, 318-322.
3. Sharma, A.; Mohanty, D.; Desai, A.; Ali, R. ***Electrophoresis.*** 2003, 24, 2733-2739.
4. Nguyen, V.; Yoshida, W.; Cohen, Y. ***J. Appl. Polym. Sci.*** 2003, 87, 300-310.

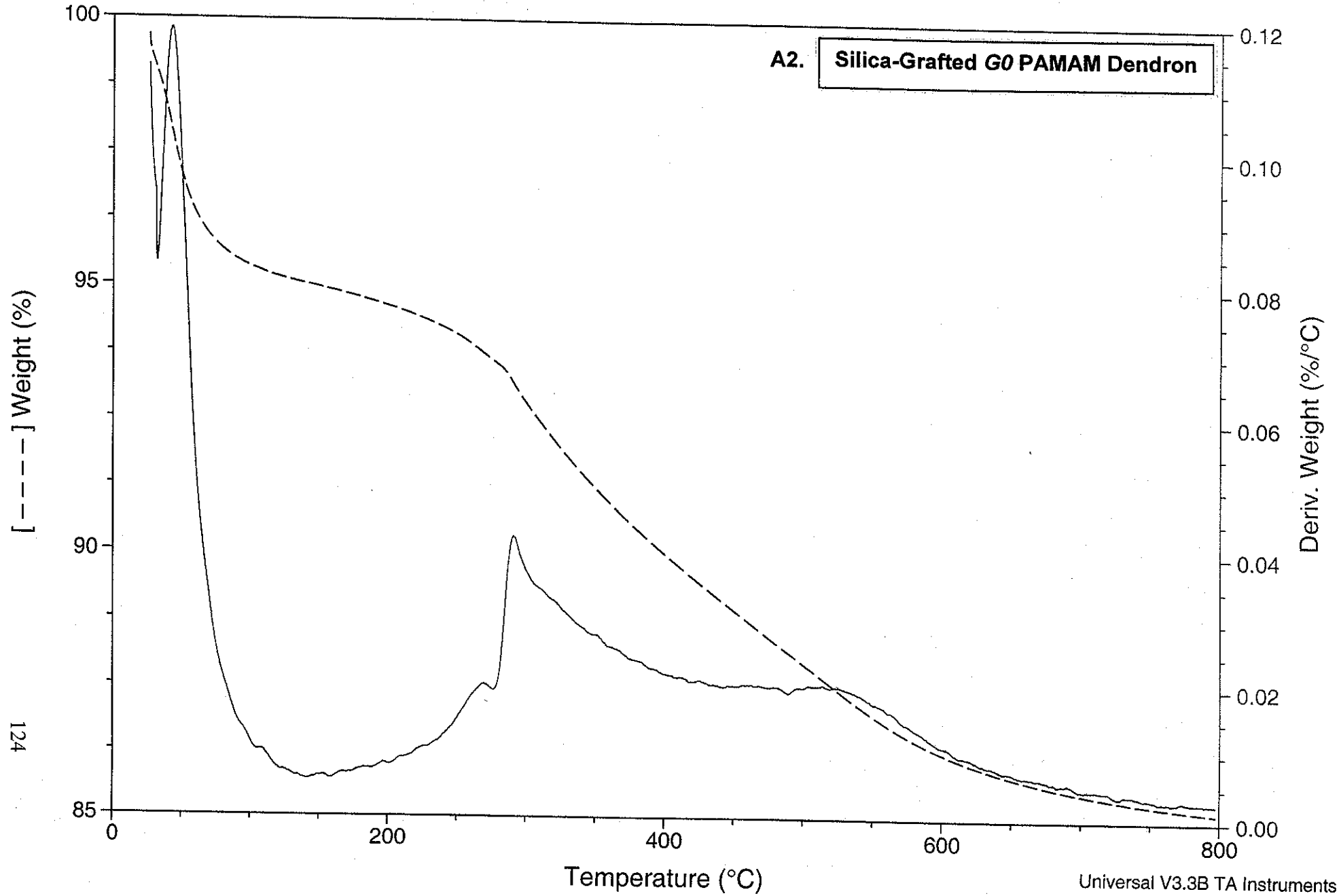
Appendixes

TGA-DTA

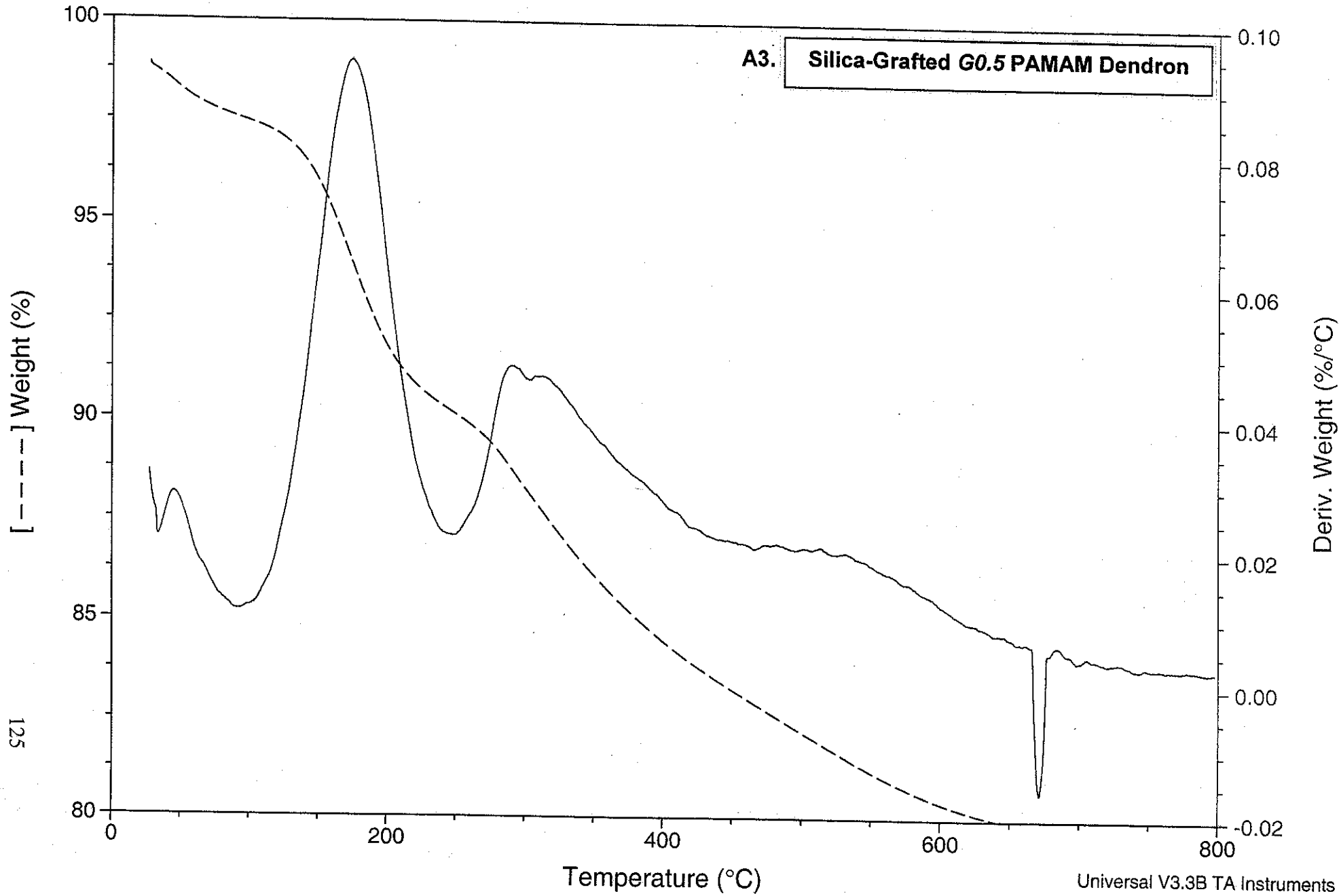

A1.
Bare Silica Gel
Spherical Silica Gel (SiliCycle)
Specifications
Particle Size: 5 Microns
Pore Diameter: 60 Angstrom
Surface Area : 500 m2/g
100
98
96
94
92
90
88
[- - - -] Weight (%)
0.15
0.10
0.05
0.00
Deriv. Weight (%/°C)
0
200
400
600
800
Temperature (°C)
Universal V3.3B TA Instruments

123

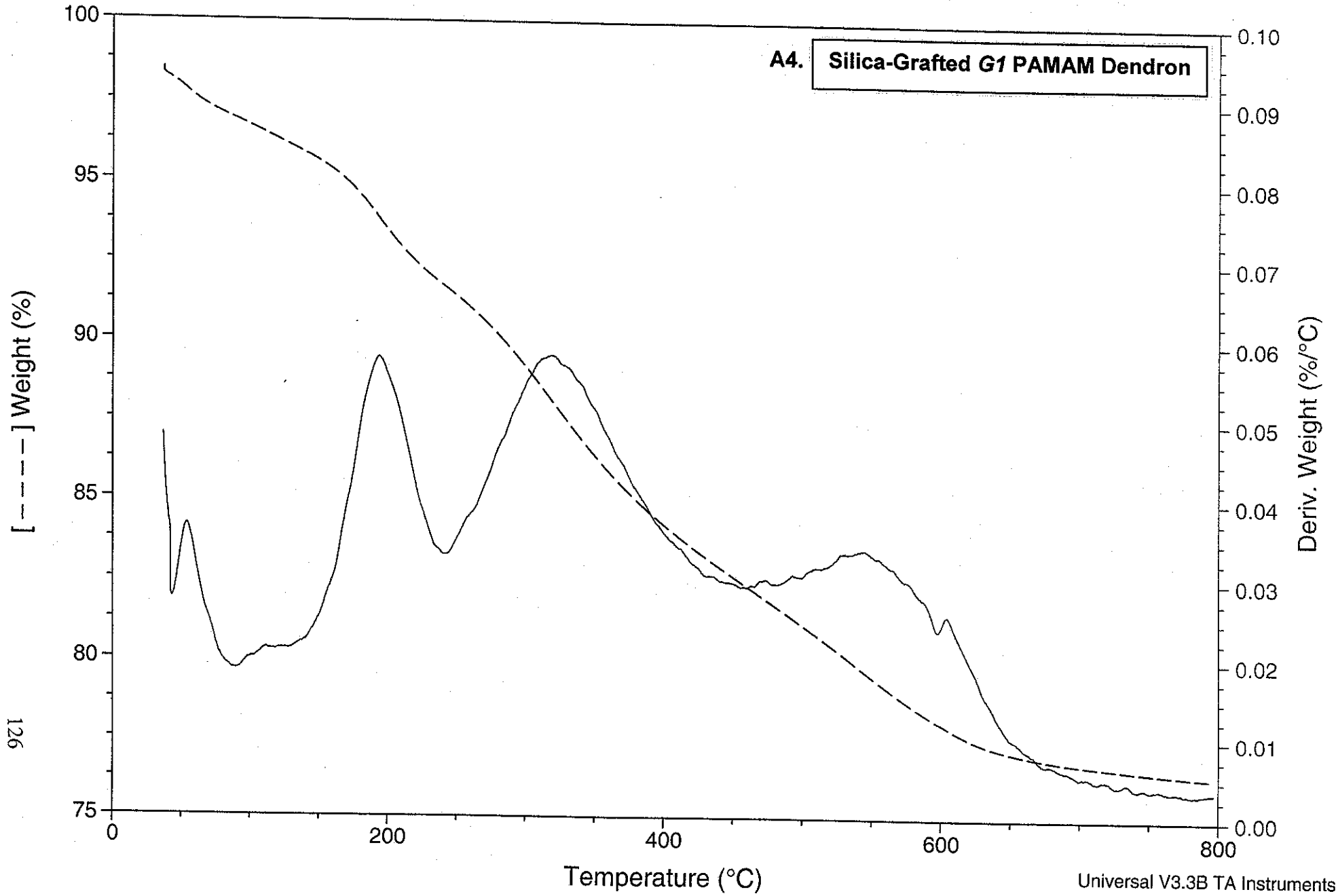
TGA-DTA


A2. Silica-Grafted G0 PAMAM Dendron
100
95
90
85
[- - - -] Weight (%)
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Deriv. Weight (%/°C)
0
200
400
600
800
Temperature (°C)
Universal V3.3B TA Instruments

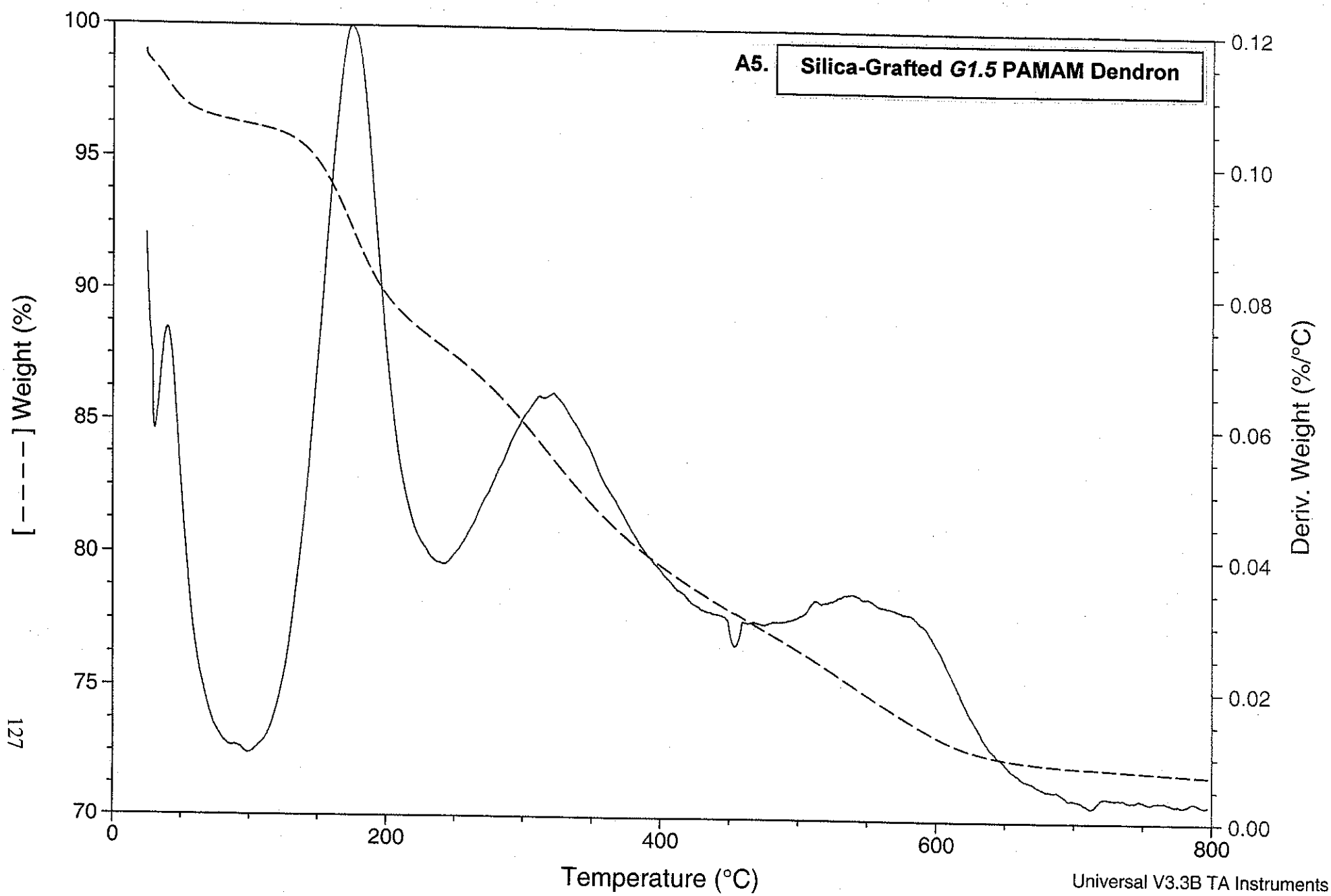
TGA-DTA


A3. Silica-Grafted G0.5 PAMAM Dendron
100
95
90
85
80
[- - - -] Weight (%)
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
Deriv. Weight (%/°C)
0
200
400
600
800
Temperature (°C)
Universal V3.3B TA Instruments

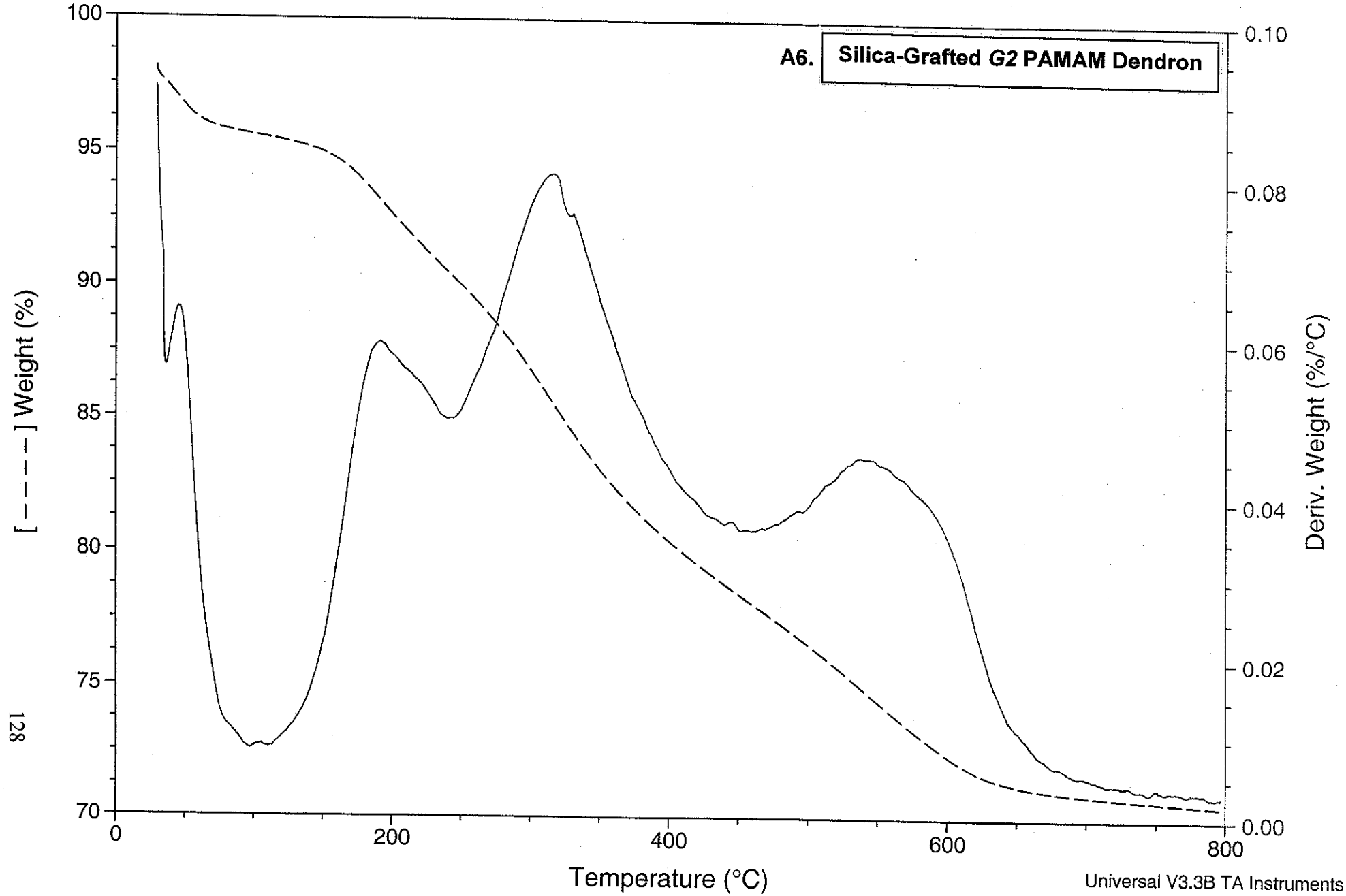
TGA-DTA


A4. Silica-Grafted G1 PAMAM Dendron
100
95
90
85
80
75
[- - - -] Weight (%)
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
Deriv. Weight (%/°C)
0
200
400
600
800
Temperature (°C)
Universal V3.3B TA Instruments

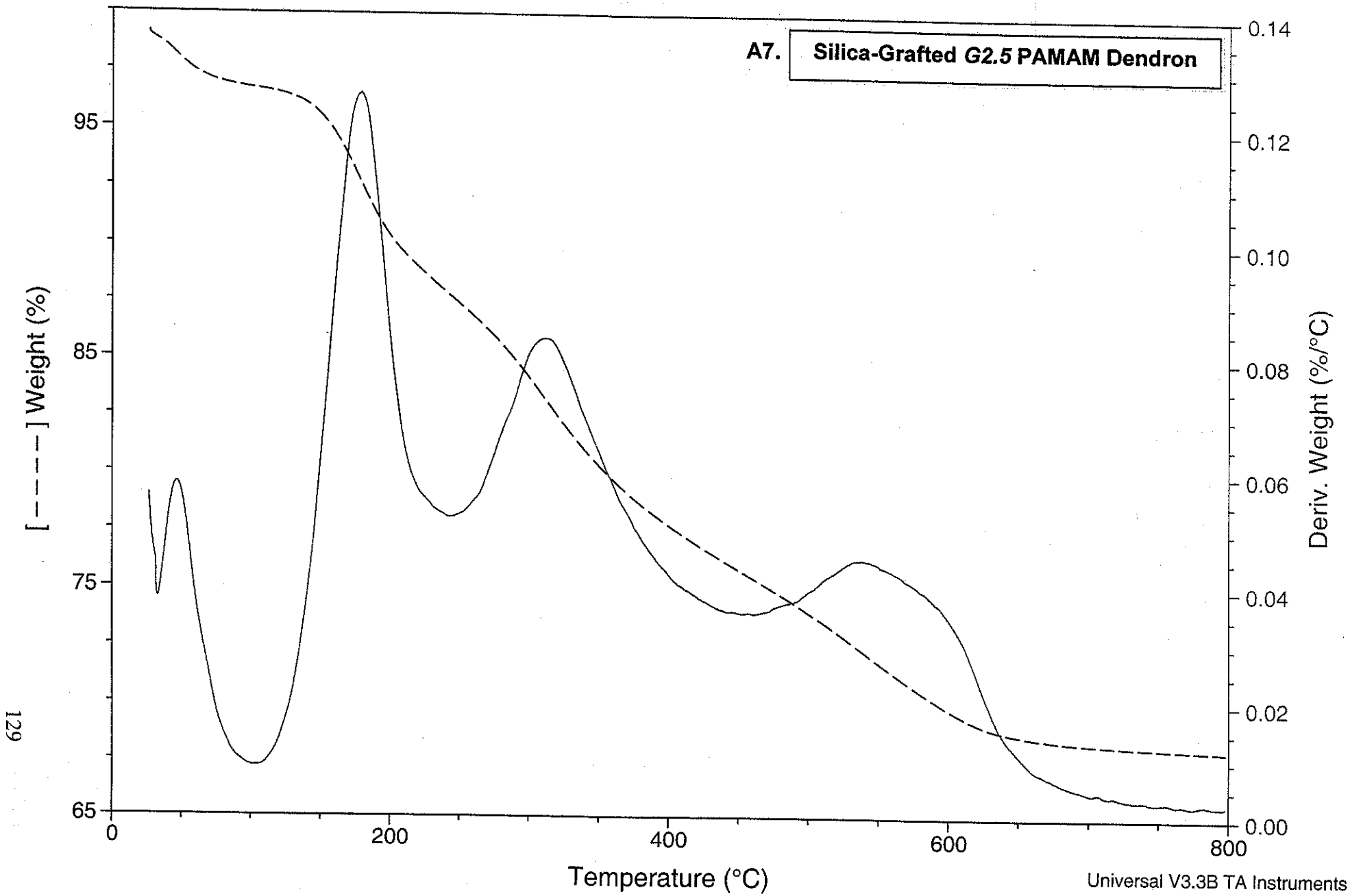
TGA-DTA


A5. Silica-Grafted G1.5 PAMAM Dendron
[- - - -] Weight (%)
Deriv. Weight (%/°C)
Temperature (°C)
100
95
90
85
80
75
70
0
200
400
600
800
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Universal V3.3B TA Instruments

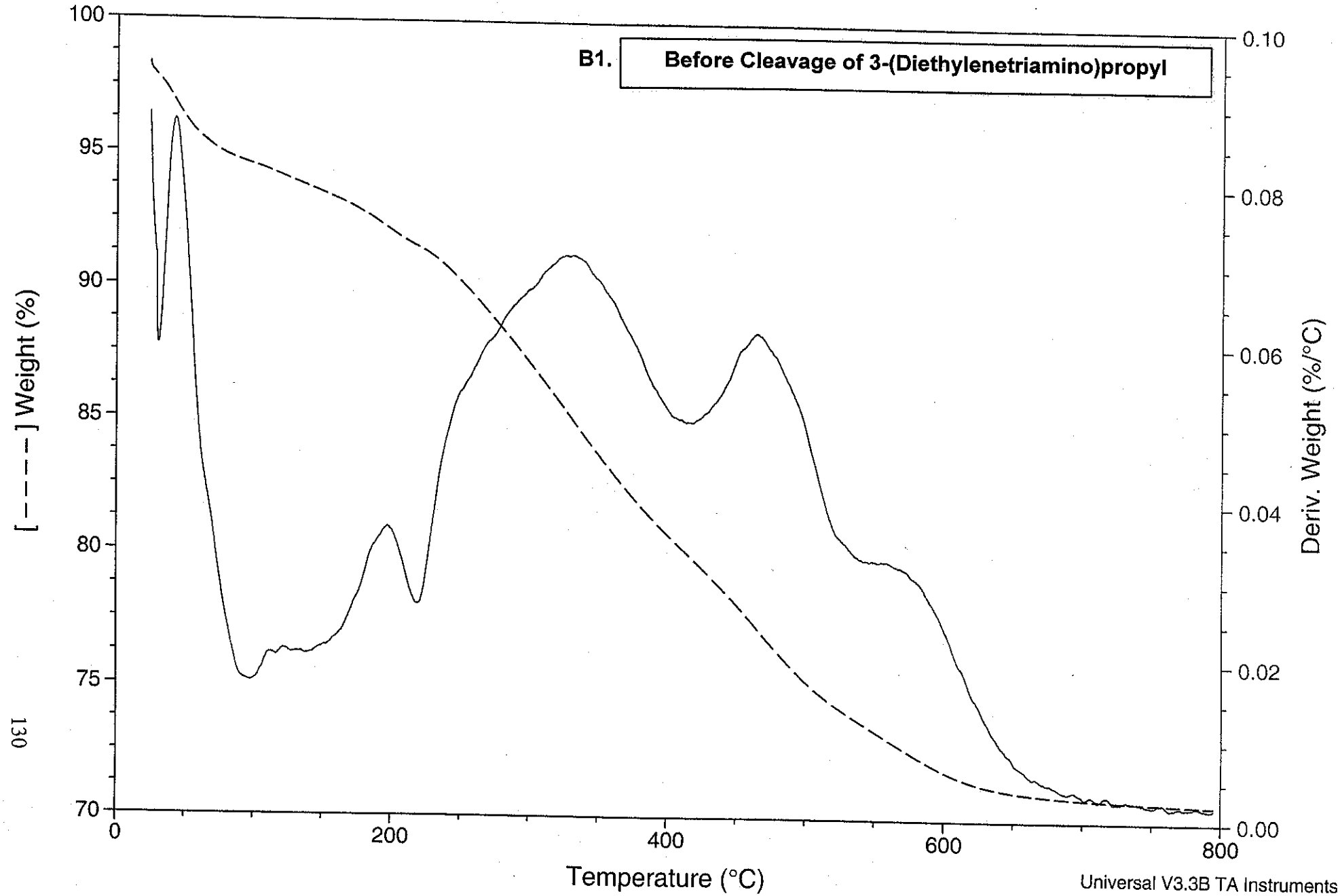
TGA-DTA


A6. Silica-Grafted G2 PAMAM Dendron
100
95
90
85
80
75
70
[- - - -] Weight (%)
0.10
0.08
0.06
0.04
0.02
0.00
Deriv. Weight (%/°C)
0
200
400
600
800
Temperature (°C)
Universal V3.3B TA Instruments

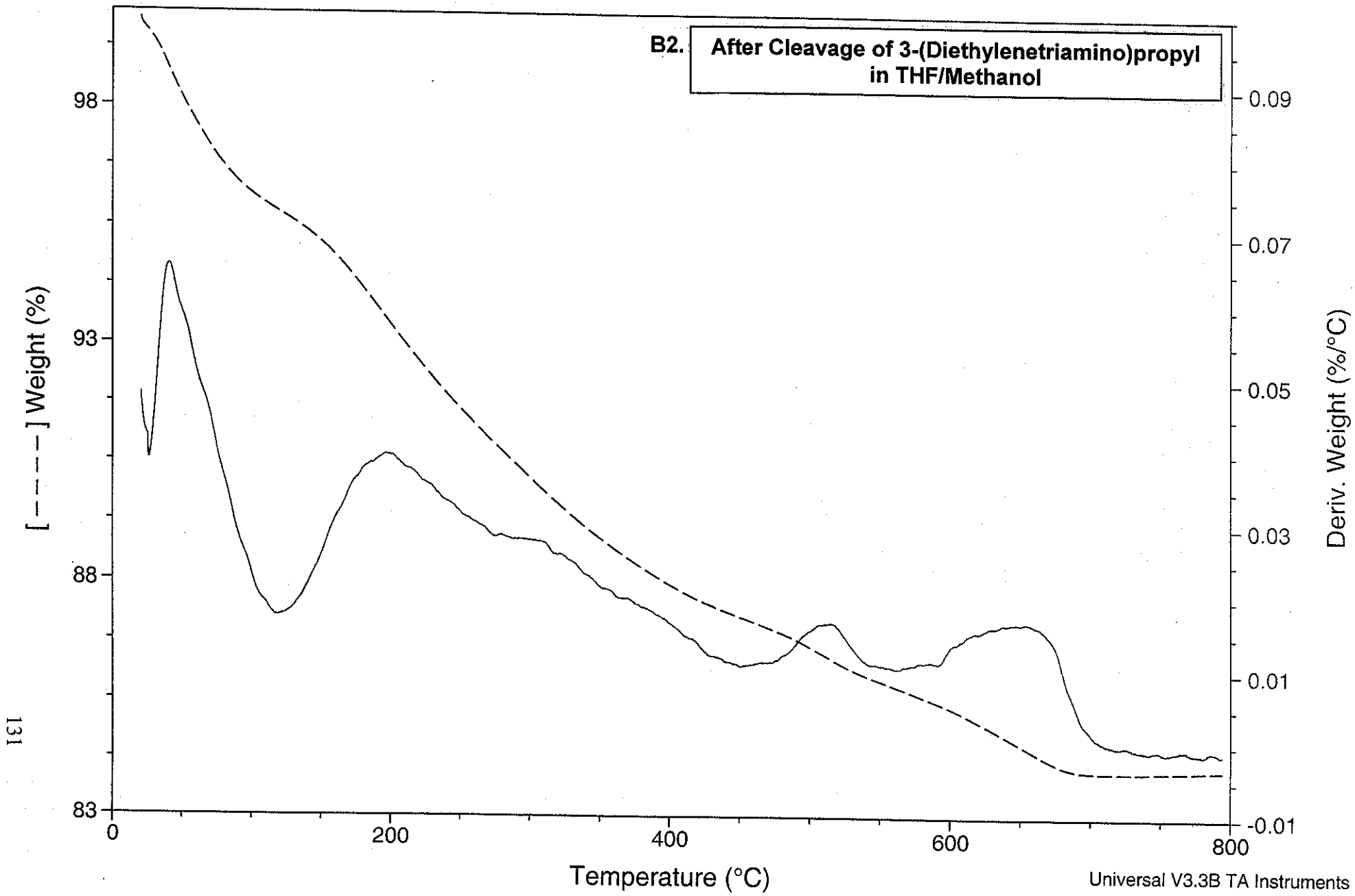
TGA-DTA


A7. Silica-Grafted G2.5 PAMAM Dendron
95
85
75
65
[- - - -] Weight (%)
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Deriv. Weight (%/°C)
0
200
400
600
800
Temperature (°C)
Universal V3.3B TA Instruments

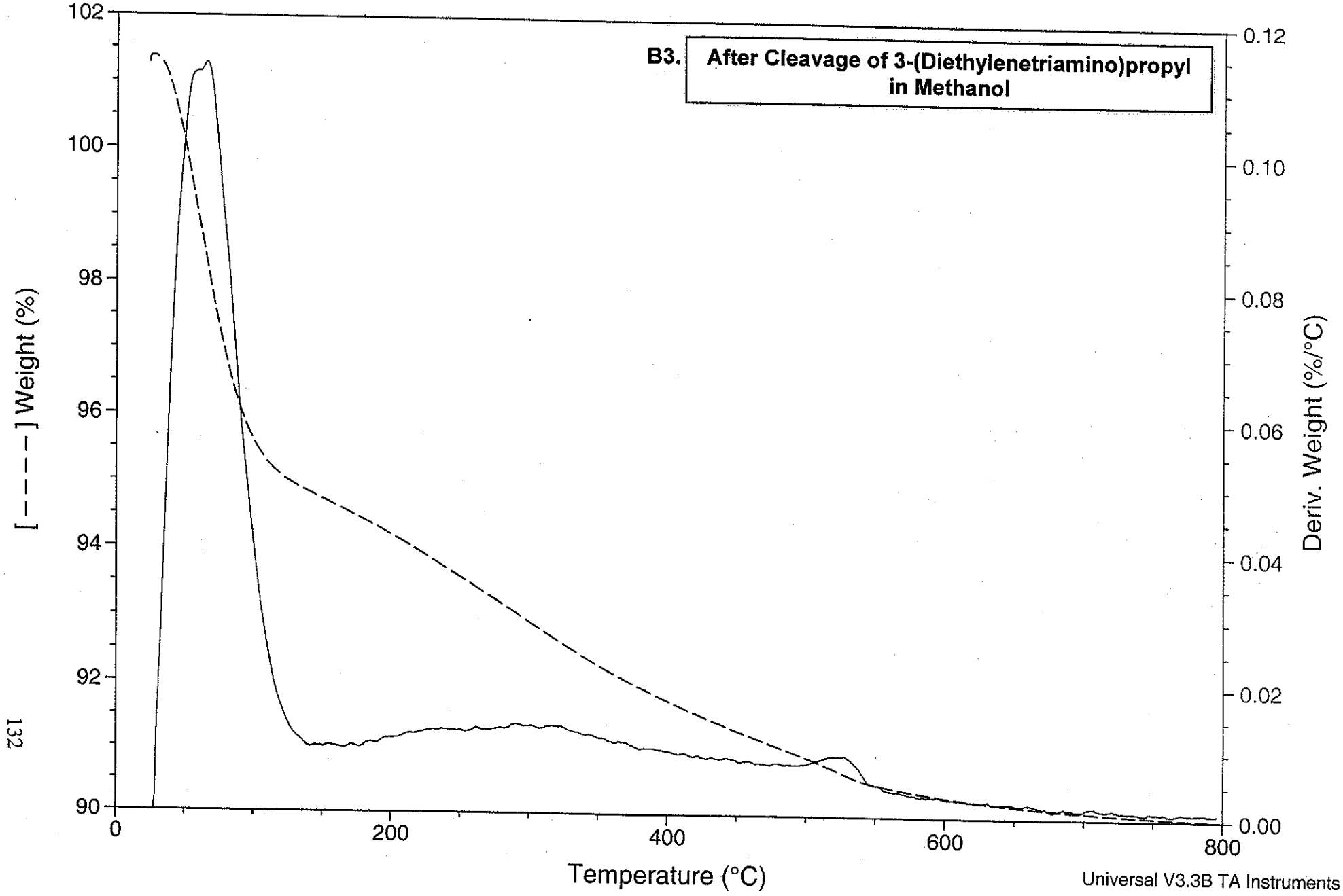
TGA-DTA


B1.
Before Cleavage of 3-(Diethylenetriamino)propyl
100
95
90
85
80
75
70
[- - - -] Weight (%)
0.10
0.08
0.06
0.04
0.02
0.00
Deriv. Weight (%/°C)
0
200
400
600
800
Temperature (°C)
Universal V3.3B TA Instruments

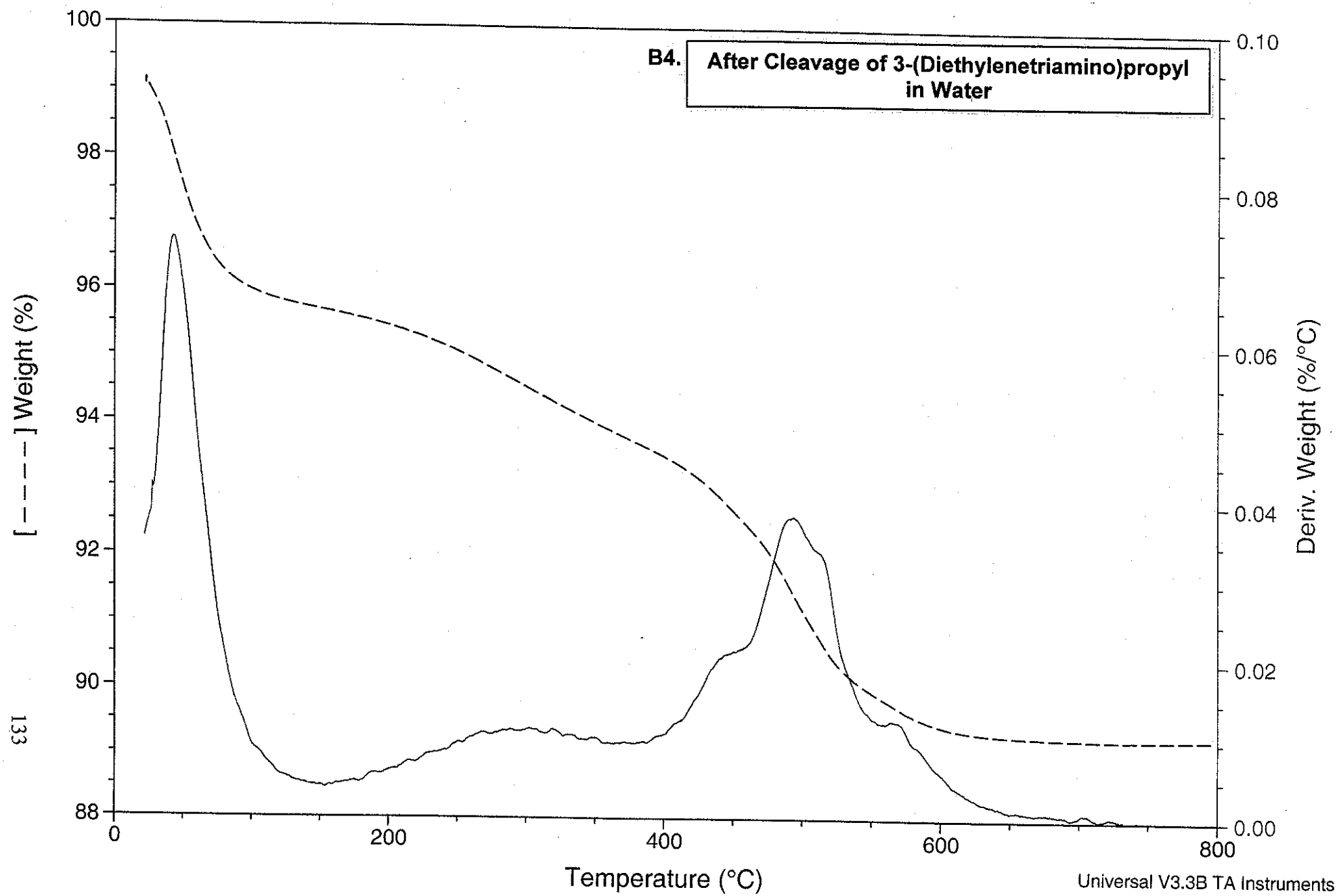
TGA-DTA


B2.
After Cleavage of 3-(Diethylenetriamino)propyl in THF/Methanol
98
93
88
83
[- - - -] Weight (%)
0.09
0.07
0.05
0.03
0.01
-0.01
Deriv. Weight (%/°C)
0
200
400
600
800
Temperature (°C)
Universal V3.3B TA Instruments

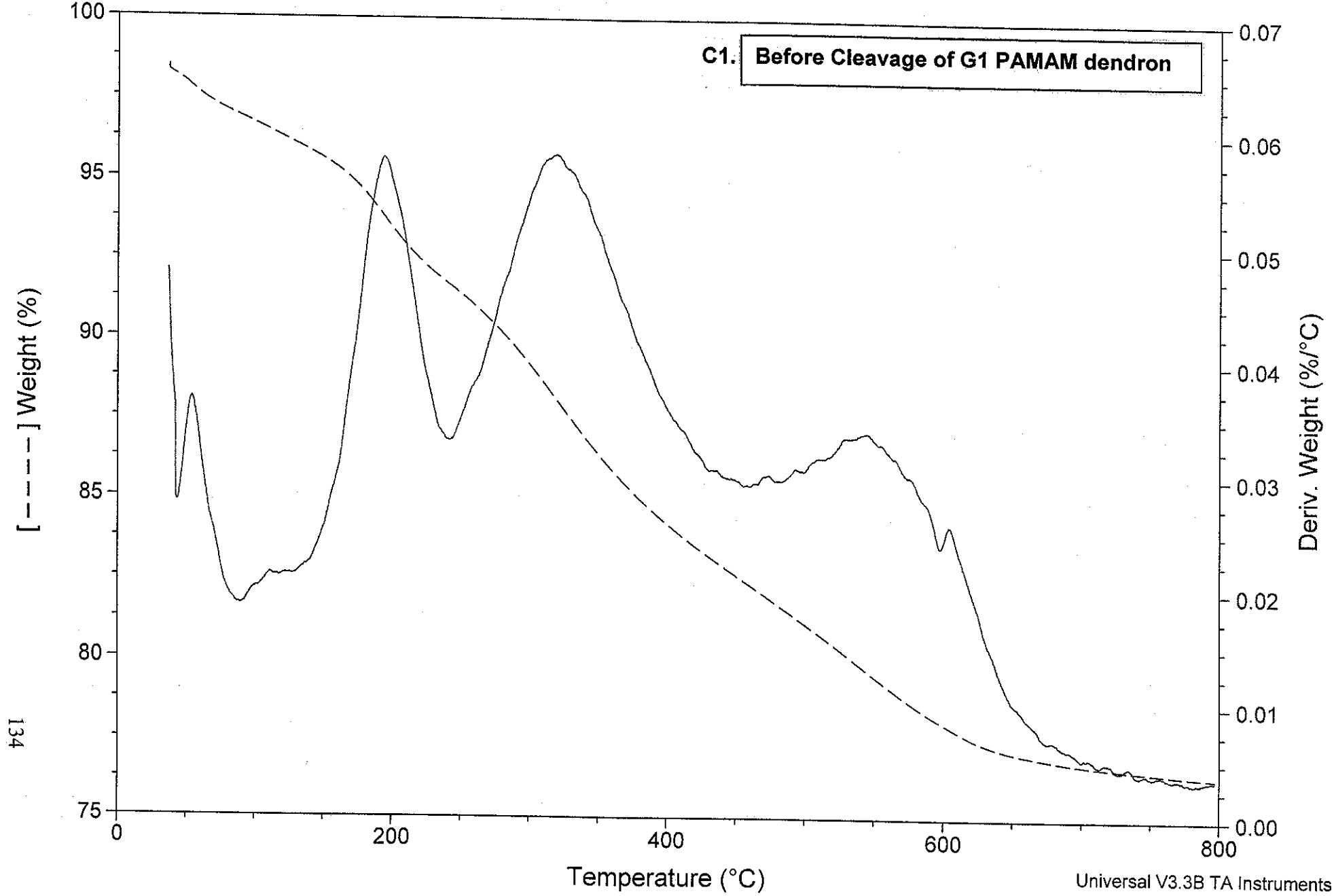
TGA-DTA


B3. After Cleavage of 3-(Diethylenetriamino)propyl in Methanol
102
100
98
96
94
92
90
[- - - -] Weight (%)
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Deriv. Weight (%/°C)
0
200
400
600
800
Temperature (°C)
Universal V3.3B TA Instruments

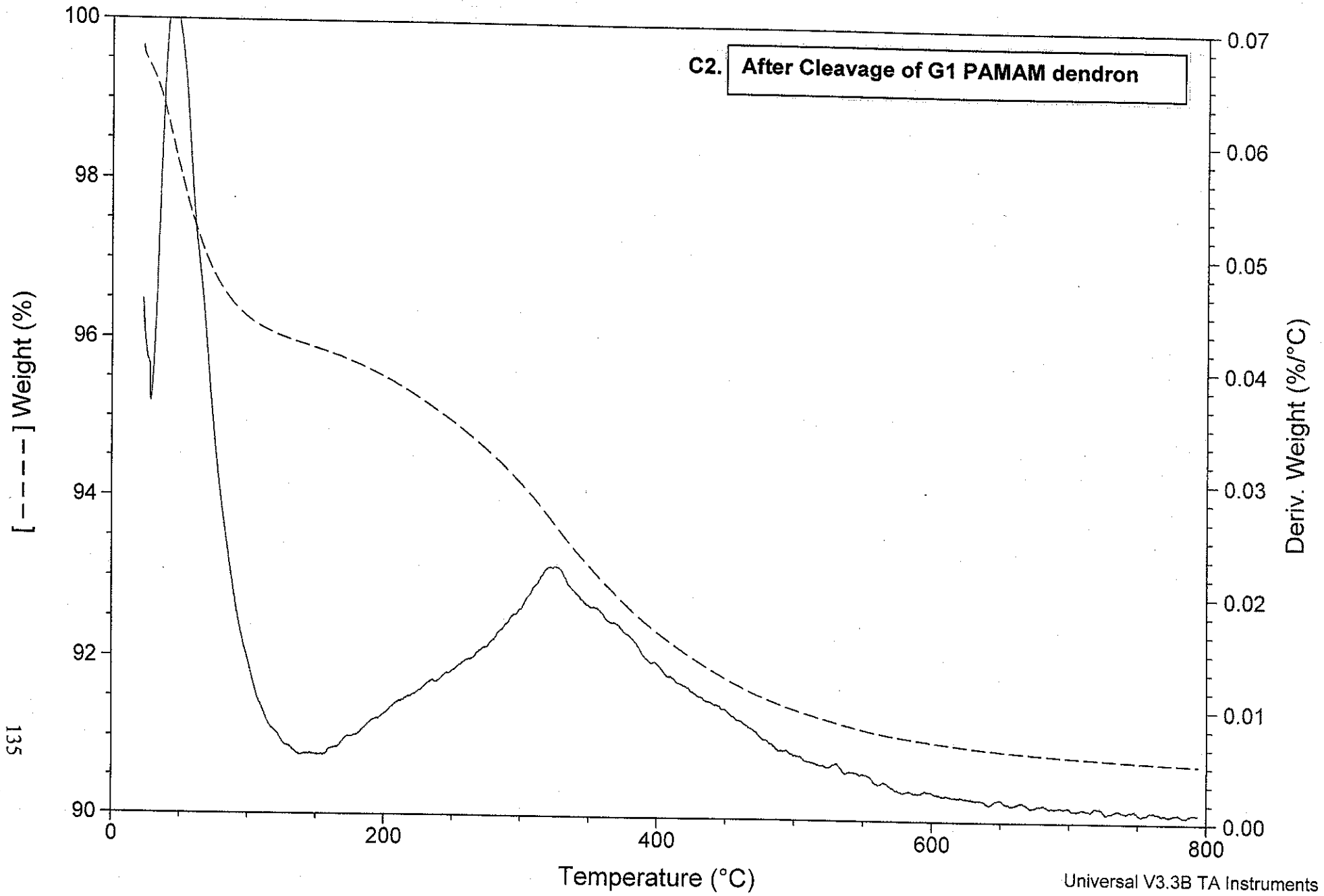
TGA-DTA


B4.
After Cleavage of 3-(Diethylenetriamino)propyl in Water
100
98
96
94
92
90
88
[- - - -] Weight (%)
0.10
0.08
0.06
0.04
0.02
0.00
Deriv. Weight (%/°C)
0
200
400
600
800
Temperature (°C)
Universal V3.3B TA Instruments

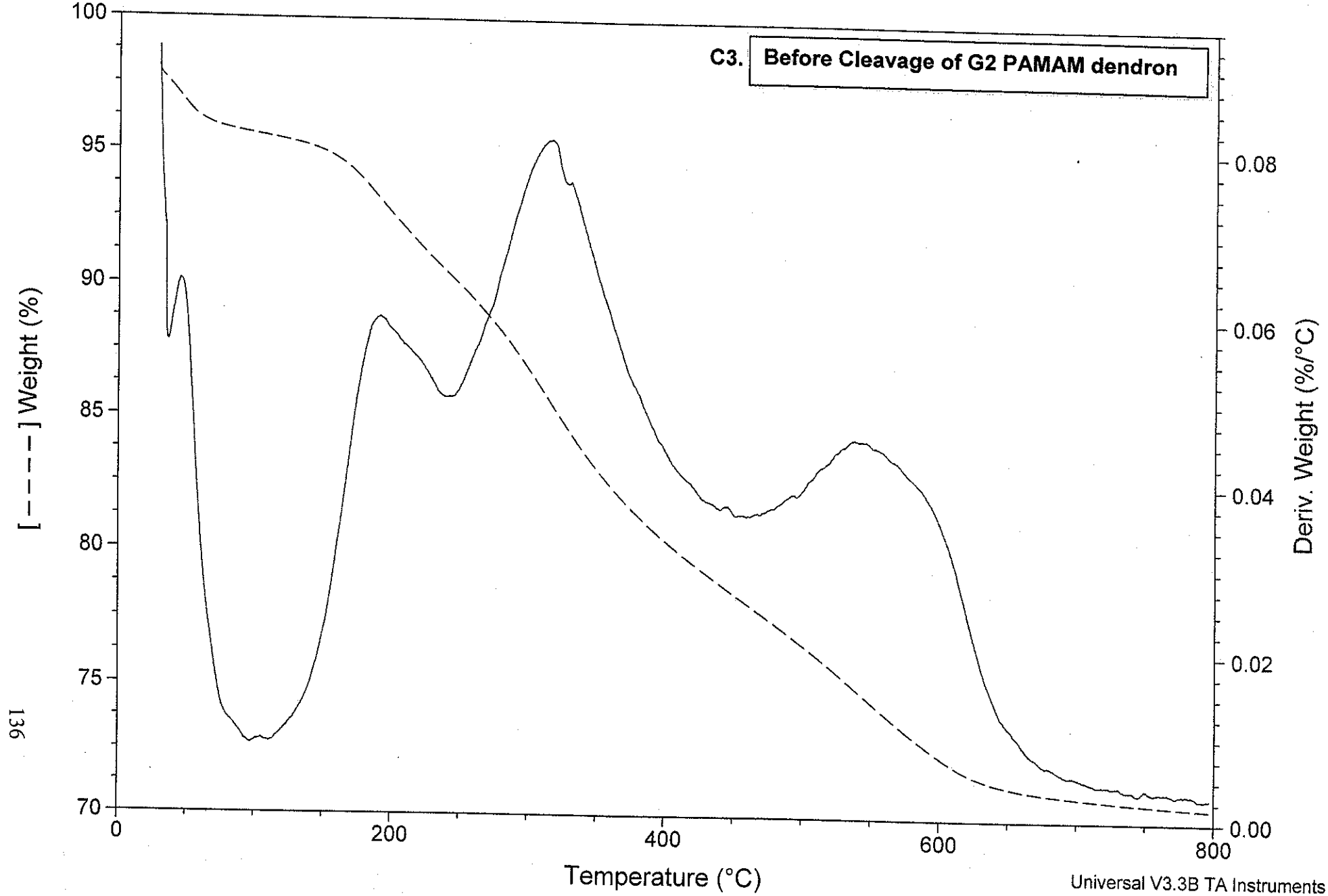
TGA-DTA


C1. Before Cleavage of G1 PAMAM dendron
100
95
90
85
80
75
[- - - -] Weight (%)
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
Deriv. Weight (%/°C)
0
200
400
600
800
Temperature (°C)
Universal V3.3B TA Instruments

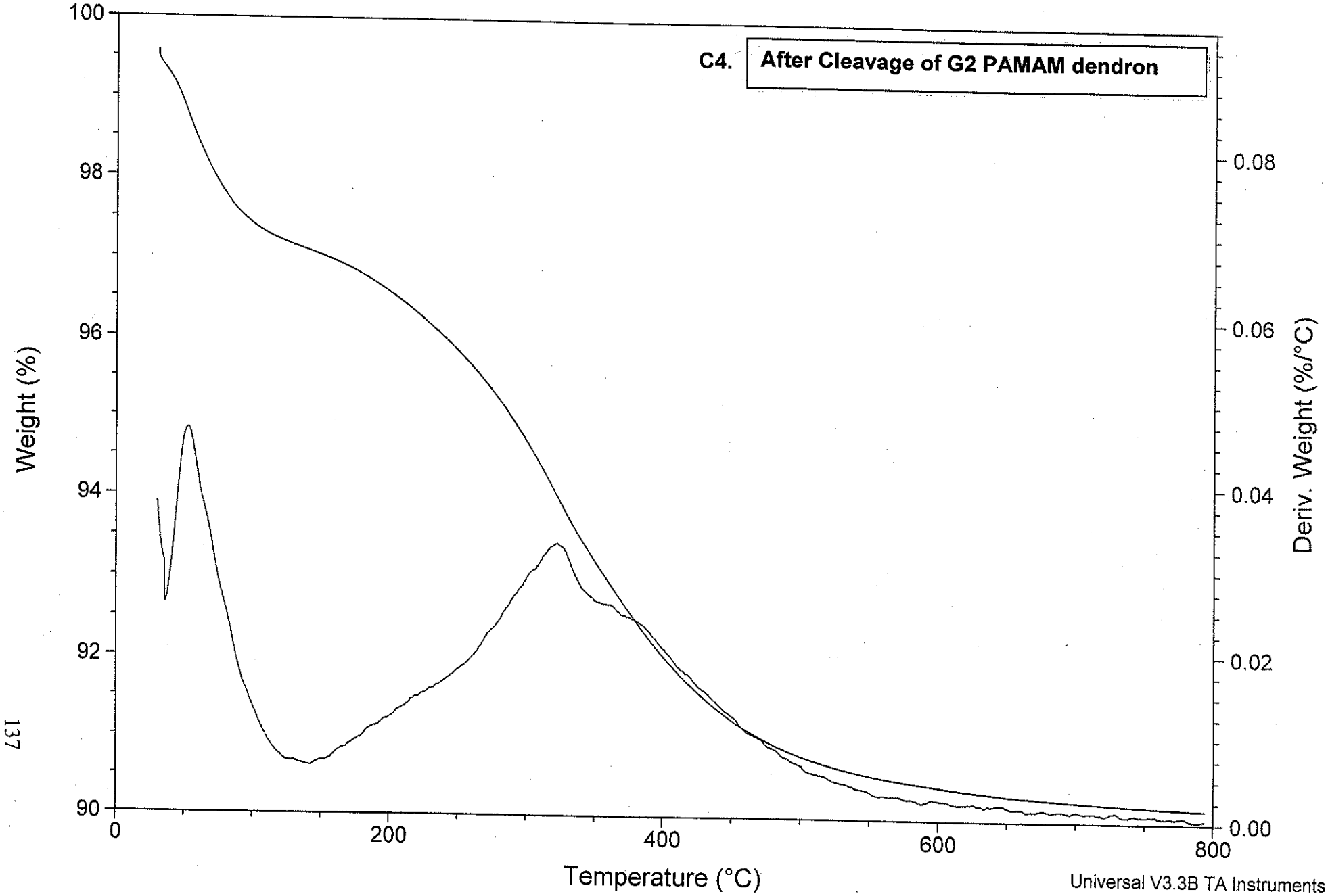
TGA-DTA


C2. After Cleavage of G1 PAMAM dendron
100
98
96
94
92
90
[- - - -] Weight (%)
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
Deriv. Weight (%/°C)
0
200
400
600
800
Temperature (°C)
Universal V3.3B TA Instruments

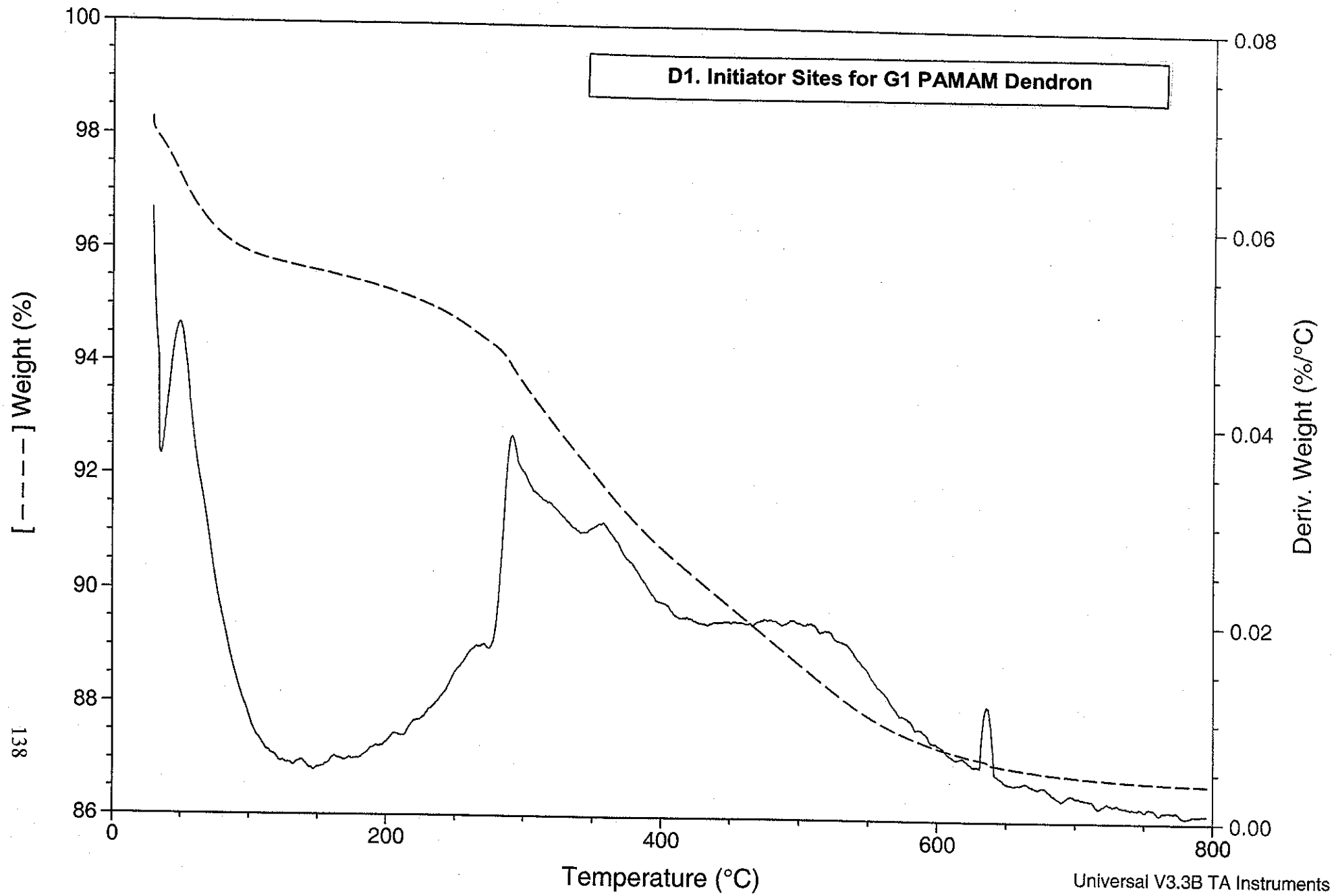
TGA-DTA


C3. Before Cleavage of G2 PAMAM dendron
100
95
90
85
80
75
70
[- - - -] Weight (%)
0.08
0.06
0.04
0.02
0.00
Deriv. Weight (%/°C)
0
200
400
600
800
Temperature (°C)
Universal V3.3B TA Instruments

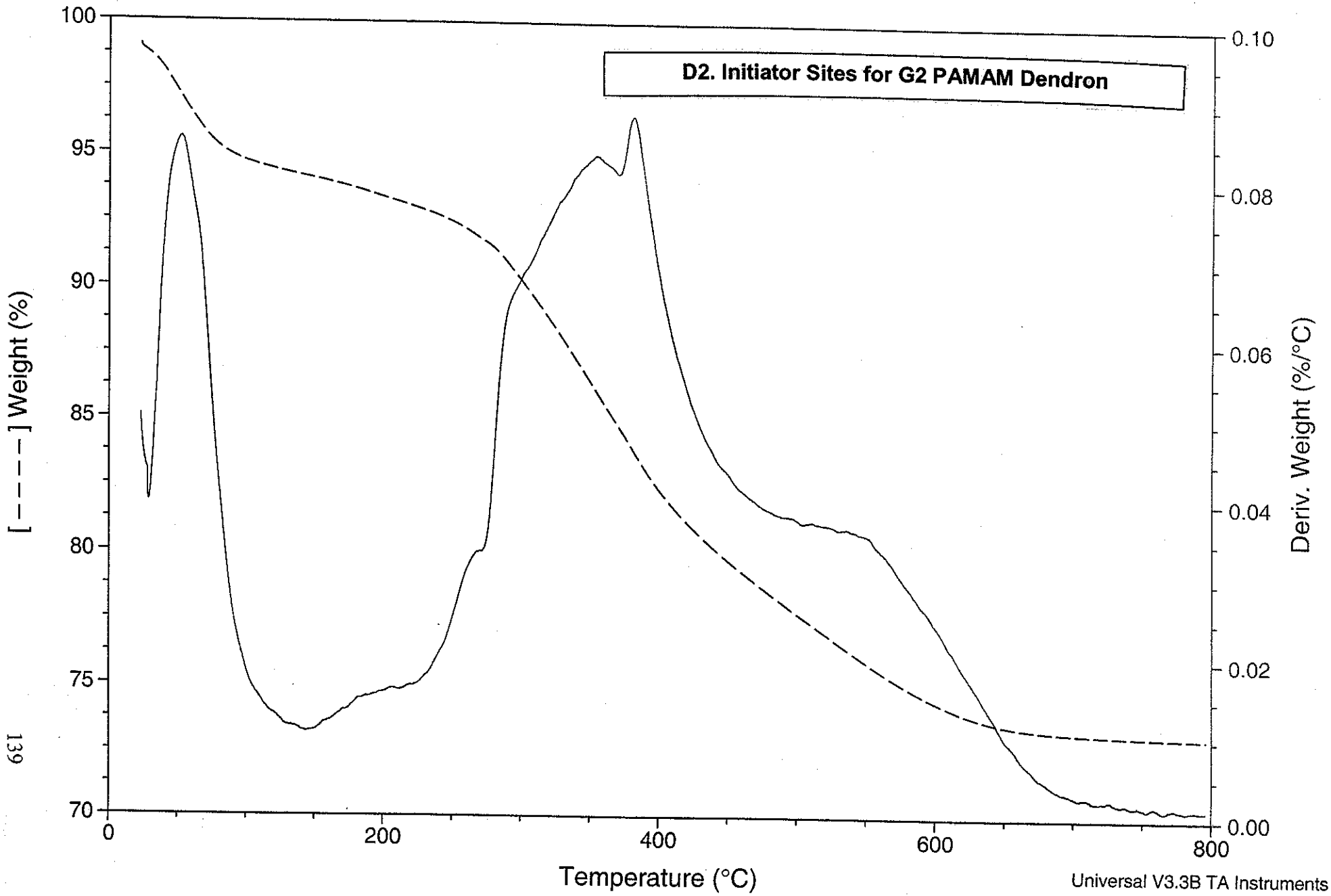
TGA-DTA


C4. After Cleavage of G2 PAMAM dendron
100
98
96
94
92
90
Weight (%)
0.08
0.06
0.04
0.02
0.00
Deriv. Weight (%/°C)
0
200
400
600
800
Temperature (°C)
Universal V3.3B TA Instruments

TGA-DTA


D1. Initiator Sites for G1 PAMAM Dendron
[- - - -] Weight (%)
Deriv. Weight (%/°C)
Temperature (°C)
100
98
96
94
92
90
88
86
0.08
0.06
0.04
0.02
0.00
0
200
400
600
800
Universal V3.3B TA Instruments

TGA-DTA


D2. Initiator Sites for G2 PAMAM Dendron
100
95
90
85
80
75
70
[- - - -] Weight (%)
0.10
0.08
0.06
0.04
0.02
0.00
Deriv. Weight (%/°C)
0
200
400
600
800
Temperature (°C)
Universal V3.3B TA Instruments