ANION RECOGNITION PROPERTIES OF POLY(AMIDOAMINE) DENDRIMER FUNCTIONALIZED WITH HAMILTON RECEPTOR MOIETY IN AQUEOUS SOLUTIONS CONTAINING NITRATE AND NITRITE IONS

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Specially dedicated to my parent, Rusli bin Mohd Zain and Zainab Binti Ismail, brothers, sisters and dearest friends.

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PREFACE

This thesis is the results of my work carried out in the Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, between September 2010 to January 2013 under supervision of Prof. Dr. Salasiah Endud. Parts of my work described in this thesis have been reported in the following publications or presentations:

1. Endud, S. and Rusli, R., "Synthesis of Poly(amidoamine)-Dendrimer-Silver Nanocomposites for Application as Bacteriocides", Proceedings International Conference on the Advancement of Materials and Nanotechnology II (ICAMN II), Prince Hotel & Residence, Kuala Lumpur, 29th Nov – 2nd Dec 2010.

2. Endud, S., Chien, L. S., Mohd Nesfu, N. Z., Othman, A. S., Golbaha, N. and Rusli, R., "DENs-P, Polyfunctional Dendrimer Encapsulated Nanoparticles", Silver Medal Winner at the Industrial Art and Technology Exhibition 2011 (INATEX 2011), Universiti Teknologi Malaysia, 16th – 18thNov. 2011.

3. Endud, S., Ramli, Z. and Rusli, R., "Synthetic Macrocylic Hamilton Receptor Derivative Comprising Amide Group". Oral presentation at the *24th Regional Symposium of Malaysia Analytical Sciences* (SKAM 24th). One Helang Hotel, Langkawi, organized by Universiti Teknologi MARA and Malaysian Analytical Sciences Society (ANALIS), 21st – 23rd November 2011.

4. Endud, S., Chien, L., S., Mohd Nesfu, N., Z., Othman, A. S., Golbaha, N. and Rusli, R., "Dendrimer Engineered Nanoparticles", Gold Medal Winner, Seoul International Invention Fair 2011 (SIIF 2011), Seoul, Korea, 1^{st} – 4^{th} Dec 2011.

5. Endud, S., Mohd Nesfu, N. Z., Rusli, R. and Othman, A. S., "DENs-P, Polyfunctional Dendrimer Encapsulated Nanoparticles with Antibacterial Activity", Silver Medal Winner, at Malaysia Technology Expo 2012, (MTE 2012), Kuala Lumpur, 16^{th} – 18^{th} Feb. 2012.

ABSTRACT

Poly(amidoamine) generation 0.5 (PAMAM G0.5) was functionalized at the peripheral with N^1,N^3-bis(6-(3,3-dimethylbutanamido)pyridine-2-1yl)-5-amino benzene-1,3-diamide, denoted as Hamilton receptor, for anion recognition studies of nitrate and nitrite ions in aqueous solutions. The PAMAM dendrimers were prepared by divergent method using ethylenediamine and methyl acrylate via Michael addition process. The four amino end groups of 2,6-diaminopyridine were used as precursors that bind the Hamilton receptor moieties which subsequently were conjugated to the ester terminal groups of PAMAM G0.5 at a 4:1 molar ratio to give Hamilton-receptor-functionalized PAMAM G0.5, HR-PAMAM G0.5. All the materials were characterized by Fourier transform infrared (FTIR) and proton nuclear magnetic resonance (^{1}H NMR) spectroscopies. Fluorescence spectroscopy together with fluorescence quenching method were used to estimate the binding association constants (K_{ass}) of HR-PAMAM G0.5 towards specific anions such as monoanions (i.e. F^-, Br^-, Cl^-) and oxyanions (i.e. NO_3^-, NO_2^-, AcO^-) in aqueous solutions. As anion recognition material, HR-PAMAM G0.5 was able to discriminate between the anions as shown by fluorescence spectra. The results of binding constant measurements indicate that it did not form hydrogen bonds with halide or acetate anion but exhibited excellent selectivity for trigonal planar oxyanions such as nitrate (NO_3^-) or nitrite (NO_2^-). The HR-PAMAM G0.5 receptor demonstrated substantially higher affinity for NO_3^- than NO_2^- anion suggesting much stronger multiple hydrogen bonding interactions occurred between the Hamilton receptor and nitro groups (-NO) of nitrate anion. In addition, the K_{ass} measured at various pH (2 – 12) showed higher values in basic environment compared to acidic due to deprotonation of amine groups of HR-PAMAM G0.5 receptor. More significantly, the HR-PAMAM G0.5 also showed binding affinity for nitrate anion in aprotic solvent such as acetonitrile by hydrogen bonding through π-electron acceptor group of the nitrile. The limit of detection of HR-PAMAM G0.5 for NO_2^- (2.85×10^{-5} M) was relatively lower than NO_3^- (4.06×10^{-5} M) signifying greater sensitivity for the latter anion. The use of dendrimer molecule as host is advantageous for it has the ability to concentrate anion substrates which amplify the resulting fluorescent signal, as well as to provide the optimal binding conformation for hydrogen bonding with various target anions.

ABSTRAK

Poli(amidoamina) generasi 0.5 (PAMAM G0.5) telah difungsikan pada bahagian luar dengan *N*[1], *N*[3]-bis(6-(3,3-dimetilbutanamido)piridin-2-lil)-5-aminobenzena-1,3-diamida dikenali sebagai reseptor Hamilton, bagi kajian pengenalan anion nitrat dan nitrit dalam larutan akueus. Dendrimer PAMAM telah disintesis dengan kaedah pencapahan menggunakan etilenadiamina dan metil akrilat melalui proses penambahan Michael. Keempat-empat kumpulan hujung 2,6-diaminopiridina telah digunakan sebagai pemula bagi mengikat gugusan reseptor Hamilton yang kemudian berkonjugat dengan kumpulan ester terminal PAMAM G0.5 dalam nisbah molar 4:1 bagi menghasilkan reseptor Hamilton-berfungsikan PAMAM G0.5, HR-PAMAM G0.5. Kesemua bahan telah dicirikan menggunakan spektroskopi inframerah transformasi Fourier (FTIR) dan spektroskopi resonans magnet nukleus bagi proton (^{1}H RMN). Spektroskopi pendarfluor yang diiringi dengan kaedah pelindapkejutan pendarfluor telah digunakan untuk menganggar pemalar pengikatan bersekutu, K_{ass}, HR-PAMAM G0.5 terhadap anion tertentu seperti monoanion (i.e. F^-, Br^-, Cl^-) dan oksianion (i.e. NO_3^-, NO_2^-, AcO^-). Sebagai suatu bahan pengenalan anion, HR-PAMAM G0.5 berupaya membezakan sifat optik anion yang dikaji berdasarkan spektrum pendarfluor masing-masing. Keputusan pengukuran K_{ass} menunjukkan ia tidak membentuk ikatan hidrogen dengan anion halida atau asetat tetapi memperlihatkan kepilihan tinggi terhadap oksianion trigonal bersatah seperti nitrat (NO_3^-) atau nitrit (NO_2^-). Reseptor HR-PAMAM G0.5 menunjukkan afiniti yang jauh lebih tinggi terhadap NO_3^- berbanding NO_2^-, mencadangkan terdapat pengikatan hidrogen berganda antara reseptor Hamilton dan kumpulan nitro (-NO) anion nitrat. Tambahan lagi, K_{ass} yang diukur pada pelbagai pH (2-12) menunjukkan nilai yang pada umumnya lebih tinggi dalam persekitaran bes berbanding keadaan berasid yang disebabkan oleh nyahproton kumpulan amina dari reseptor HR-PAMAM G0.5. Lebih penting lagi, HR-PAMAM G0.5 juga didapati mempunyai afiniti pengikatan terhadap anion nitrat dalam pelarut aprotik seperti asetonitril melalui pembentukan ikatan hidrogen yang melibatkan kumpulan penerima elektron-π iaitu nitril. Had pengesanan bagi HR-PAMAM G0.5 untuk NO_2^- (2.85×10^{-5} M) secara relatif lebih rendah daripada NO_3^- (4.06×10^{-5} M), menunjukkan bahawa bahan tersebut lebih sensitif terhadap pengesanan anion NO_2^-. Penggunaan molekul dendrimer sebagai perumah mempunyai kelebihan dari segi keupayaan menumpu substrat anion dalam larutan akueus sehingga menguatkan signal pendafluor yang dihasilkan. Di samping itu, struktur dendrimer yang unik juga menyediakan komformasi pengikatan hidrogen yang optimum dengan pelbagai anion sasaran.

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LIST OF ABBREVIATIONS

^{1}H NMR	Proton Nuclear Magnetic Resonance
BRB	Britton-Robinson buffer
cm^{-1}	Centimeter
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
EDA	Ethylenediamine
FTIR	Fourier Transformer Infrared
g	Gram
G	Generation
h	Hour
LOD	Limit of detection
LOQ	Limit of quantification
M	Molar
MA	Methyl acrylate
ml	Milliliter
mol	Mole
nm	Nanometer
PAMAM	Poly(amidoamine)
rt	Room temperature.
THF	Tetrahydrofuran
TBA	Tetrabutylammonium

LIST OF SYMBOLS

λ	Wavelength
%	Percent
°	Degree

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Anions such as nitrate and phosphate play critical roles in a wide range of biological processes and environmental pollutants from the use of fertilizers in the agriculture industry (Newkome *et al.,* 1985). Anions are atoms or groups of atoms that have gained electrons and they are negatively charged. Fertilizers are soil amendments applied to promote plant growth and the main nutrients present in fertilizer are nitrogen, phosphorus, and potassium. High application rates of inorganic fertilizers such as nitrogen in order to maximize crop yields combined with the high solubilities of these fertilizers leads to increased run off into surface water as well as leaching into groundwater. This condition promotes the increase of nitrate levels above 10 mg/L (10 ppm) in groundwater and leads to the 'blue baby syndrome' (acquired methemoglobinemia), leading to hypoxia (which can lead to coma and death if not treated). Because of this, anions especially nitrate and nitrite are needed to be recognized in order to effectively monitor their presence and remove them from the aqueous media.

The study of anion recognition has been a critical part in the most recent research in the area of host-guest chemistry (Reinhoudt 1995). In order to differentiate a target anion from others, the host molecule must be carefully designed, considering not only the structural complementary interaction between the ion-molecule pair, but also interactions with solvent molecules. The host molecules

for anion recognition comprise at least one interaction site that improves the selective interaction for the target anion and overcomes the solvation energy of the target anion in the aqueous phase. Examples of host molecule are, polyaza receptors, cationic cyclophanes, porphyrin and guanidium-based receptors (Tomalia *et al.*, 1985). Among these host molecules, dendrimers are of considerable interest as anion recognition material due to their fascinating structure and unique catalytic, optical, chemical, sensor and electrochemical properties (Klajnert and Bryszewska, 2001).

Dendrimer is a branched polymer having core, internal cavities, interior group and surface groups that can be modified (Dirksen *et al.*, 2004). Other than that, dendrimer had been proven to be a good host material for metal nanoparticles as well as other stabilizing agents such as citrates, organic solvents (THF or THF/MeOH), long chain alcohols, surfactants, and organometals in organic solvents (Zakaria *et. al*., 2009). These characteristics, along with water solubility, are some of the features that make them attractive for environmental remediation (Newkome *et al.*, 1985). Nowadays, there are more than fifty families of dendrimer with their own unique properties were invented for various applications (Bosman *et al.*, 1999). Therefore, many possible researches could be done based on the different specific properties offered by various dendrimers. The dendrimers has been widely developed in drugs delivery research (Li *et al.*, 2003), catalyst (Delort *et al.*, 2003), low electric materials (Lee *et al.*, 2005), sensor (Mynar *et al.*, 2006), single-wall carbon nanotubes (Amama *et al.* 2006), light harvesting materials (Lo and Burn, 2007) and host-guest chemistry (Hu *et al.*, 2010).

Therefore poly(amidoamine) (PAMAM) dendrimer is proposed to be used in this anion recognition study (Figure 1.1). This type of dendrimer having amide group in the interior cavity and amine (full generation) or ester (half generation) at the terminal, are easily modified and can form hydrogen bonding with other molecule. This characteristic is suitable to trap the anions that have either oxygen, hydrogen, nitrogen and fluorine atom.

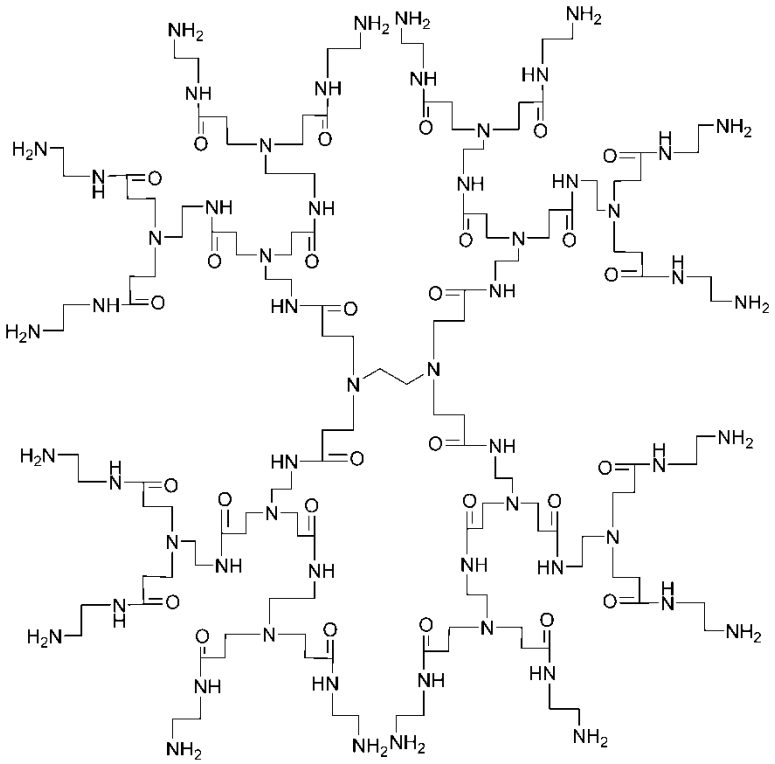

Figure 1.1: PAMAM dendrimer (generation 3.0)

Besides, addition of receptor at the periphery of dendrimer is expected to increase the complexation ability and the selectivity of anion recognition. Receptor is a molecule that can binding tightly with other molecule and act as a host guest for the molecule. In this study, the Hamilton receptor is highly encouraged to be the functional group at the periphery of the PAMAM dendrimer because its have six nitrogen atoms that can be proton donors for anions to form a covalent bonding (Klajnert and Bryszewska, 2001).

The early model of Hamilton receptor with propane group at the terminal arms is designed by Hamilton and Chang (1988) especially for the barbital recognition. This receptor is successful in interactions and recognizing the barbital in chloroform solution through its six hydrogen bonding sites with binding constant, 2.08×10^4 M^{-1}. Moreover, the proton nuclear magnetic resonance (^{1}H NMR) spectroscopy is used to calculate the binding constant from the downfield shift of proton. Figure 1.2 illustrates the structure of Hamilton receptor with two pyridine group and four amides at the arms of the receptor.

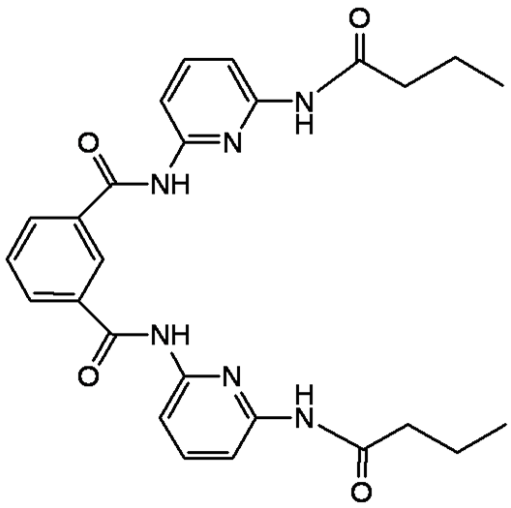

Figure 1.2: Hamilton receptor for barbital molecule.

1.2 Statement of Problem

An important source of elements such as phosphorus, sulphur and nitrogen are contained in both organic fertilizer and inorganic fertilizer (Babatunde and Ajibola, 2009). Other than that, the nitrogen element, is also widely used in the drinking water and food preservative. Nitrate and nitrite are compounds that contain a nitrogen atom joined to oxygen atoms, with nitrate containing three oxygen atoms and nitrite containing two oxygen atoms. In nature, nitrates are readily converted to nitrites and vice versa. Both are anions, or ions with a negative charge. They tend to associate with cations, or ions with a positive charge, to achieve a neutral charge balance. Nitrates are used primarily to make fertilizer, but they are also used to make glass and explosives. These compounds also are used in various chemical production and separation processes. Nitrites are manufactured mainly for use as a food preservative, and both nitrates and nitrites are used extensively to enhance the color and extend the shelf life of processed meats.

Nitrate and nitrite compounds are very soluble in water and quite mobile in the environment. They have a high potential for entering surface water when it rains, as nitrates in applied fertilizers can dissolve in run off that flows into streams or

lakes. They also have a high potential for entering groundwater through leaching. The concentration of anions associated with soil particles has been estimated to be about half the concentration in interstitial water (the water in the pore spaces between the soil particles). Nitrates themselves are relatively nontoxic. However, when swallowed, they are converted to nitrites that can react with hemoglobin in the blood, oxidizing its divalent iron to the trivalent form and creating methemoglobin. This methemoglobin cannot bind oxygen, which decreases the capacity of the blood to transport oxygen so less oxygen is transported from the lungs to the body tissues, thus causing a condition known as methemoglobinemia.

In recent research (Albretch *et al.*, (2008), Singh and Sun (2012), Weinkers *et al.*, (2012)), nitrate and nitrite anions are successfully recognized using the hydrogen bonding strategy. However, all these researches were conducted in non aqueous solutions which are chloroform, dichloromethane and dimethylsulfoxide. As a consequence, the methods adapted in those studies are generally unsuitable for detecting nitrates and nitrites in water samples. The use of the highly water soluble of PAMAM dendrimer is anticipated to assist the poor solubility of Hamilton receptor in detection of nitrate and nitrite anions in aqueous solution.

1.3 Objectives of Study

The main objectives of this study are to:

1. Synthesis PAMAM dendrimer modified with Hamilton receptor as a periphery functional group.
2. Investigate the bonding and selectivity of Hamilton receptors, PAMAM dendrimer and Hamilton receptors-PAMAM dendrimer towards various anions especially nitrate and nitrite anions.
3. Evaluate the selectivity of Hamilton receptors-PAMAM dendrimer and the effect of pH and solvents towards the binding constant (K_{ass}) of selective anions.

4. Calculate the Limit of detection (LOD) of Hamilton receptors-PAMAM dendrimer.

1.4 Scope of Study

There are three stages in this study, first is synthesizing generation 0.5 (G0.5) PAMAM dendrimer. In the second stage, the Hamilton receptor is prepared and attached to the periphery of the PAMAM dendrimer. Lastly, the modified PAMAM dendrimers are reacted with inorganic anions and the binding affinity is determined. The PAMAM dendrimer, Hamilton receptor and Hamilton recepto-PAMAM dendrimer are synthesized and characterized using FTIR and ^{1}H NMR spectroscopy prior further modifications. For the anion recognition studies, the fluorescence quenching method is used to explore the binding affinity of the Hamilton receptor-PAMAM dendrimer towards various anions in aqueous solutions. Moreover, the effect of parameters such as solvent and pH on the anion binding ability is also investigated using fluorescence spectroscopy. .

1.5 Significance of Study

This study developed a new compound of Hamilton receptor-PAMAM G0.5 dendrimer as anions recognition material especially for the nitrate and nitrite ions from the usage of inorganic fertilizer. Moreover, this study also develops an alternative way to prevent the various diseases from the effect of nitrate and nitrite anions level in water. Other than that, this study is expected to solve many problems especially in the groundwater treatment and environmental pollution.

1.6 Outline of Research

The outline of the research activities is shown in the Figure 1.3. They include synthesis, characterization and application of HR-PAMAM G0.5 as anion recognition material.

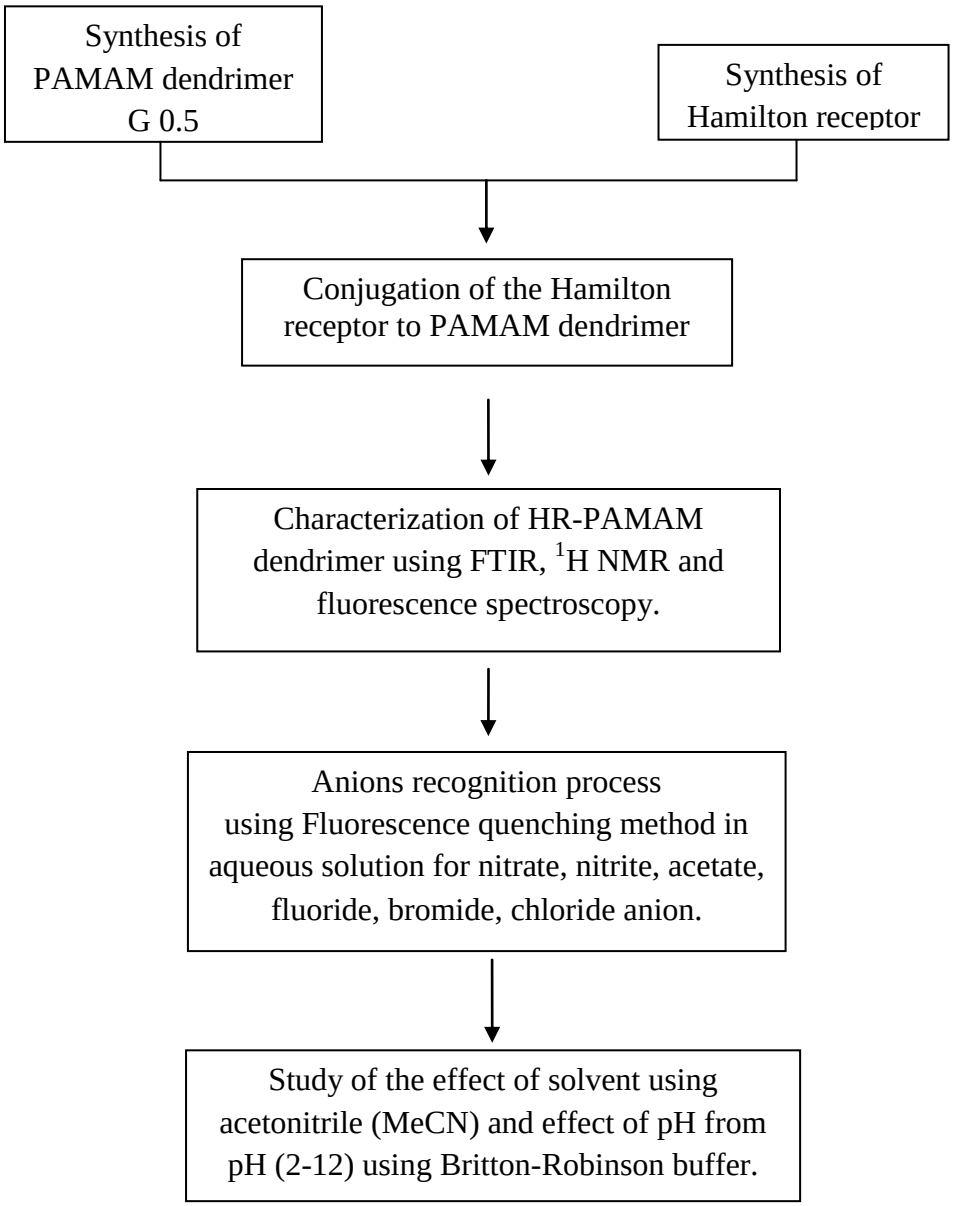

Figure 1.3: Outline of research.

1.7 Outline of Thesis

This thesis focuses on the synthesis and characterization of PAMAM dendrimer, Hamilton receptor and HR–PAMAM dendrimer. Moreover, this thesis presents the research on the capability of HR-PAMAM dendrimer to detect selectively the nitrate and nitrite anions in aqueous solutions. This thesis is arranged into five chapters as follow.

Chapter 1 describes the research background, statement of problem, objectives of study, scope of study, significance of study, outline of research and outline of thesis.

Chapter 2 discusses some literature reviews on the background dendrimers, Hamilton receptor, anion recognition of nitrate and nitrite anions and intermolecular bonding.

Chapter 3 demonstrates the materials and methods to synthesis the PAMAM dendrimer and Hamilton receptor.

Chapter 4 converses the results on synthesis and characterization of PAMAM dendrimer, Hamilton receptor and HR-PAMAM dendrimer. Additionally, it discusses the anion recognition properties of various types of anions using Hamilton receptor and Hamilton receptor-PAMAM G0.5 dendrimer in different types of solvent and at various pH.

Chapter 5 summarizes the conclusions from this research and provides some recommendation for further research.

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