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Hydroxyl Number Determination of Dendritic Polyols Utilizing NIR Spectroscopy

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Abstract

The goal of this project was to investigate the use of NIR-spectroscopy to quantify the number of OH functional groups attached to poly(amido amine) or PAMAM dendrimers in a manner that is quick, cost effective and easily reproducible. Obtaining the approximate number of hydroxyl functional groups (OH#) in a dendrimer is crucial in determining what role it can play in bonding and reactivity to other molecules. NIR-spectroscopy has the potential to allow for the recovery of the sample or at least to minimize the amount needed for analysis while providing an accurate determination of the OH# of a dendrimer.

Introduction

Dendrimers are highly functional polymers (HFPs) which are unique from other polymers because they have low polydispersity, are spherically shaped and can have a surface with many functional groups (see **Figures 1 & 2**). Dendrimers are distinguished from hyperbranched polymers because of their uniformity and manufacturing process, dendrimers have layers added sequentially, called generations, whereas hyperbranched polymers are generally made all in one synthetic step. There are other types of highly branched polymers called dendrigrafts and dendronized polymers that also fit into the dendritic family. Dendrigrafts are made by attaching branched polymers onto branched linear polymers, and dendronized polymers are the addition of portions of dendrimers to a linear polymer backbone. All of these highly branched materials try to tap into the power of polyvalent presentation of functional groups. Dendrimers and hyperbranched dendrimers are the most cost effective and commercialized technologies to date. The polyvalent presentation of functional groups provide novel properties for sticking to surfaces or in cross-linking applications. Dendrimers remain relatively expensive, but financially viable for low volume, high performance applications in research, life science reagents, and in vitro diagnostics. Dendrimers have important practical applications, such as crosslinkers, surfactants, rheology modification, signal amplification, solubility enhancement, and gene delivery applications.[1] Obtaining the approximate number of hydroxyl functional groups (OH#) attached to a particular polyol or hydroxyl-dendrimer is crucial in determining what role it can play in bonding and reactivity to other molecules. It can also be used a quality control tool to help ensure batch-to-batch consistency in dendrimer or polyol manufacturing. OH# is used by the polyurethane community to help control and tailor the cross-linking properties of polyols with isocyanates. The current standard methods employed to obtain the OH# is to calculate it from a KOH titration following application of acetic anhydride and a catalyst. This method is undesirable due to the inability to recover the sample, the time consuming nature of the process (four to sixteen hours) and results that are very difficult to reproduce even by well-trained chemists.[2-2b]

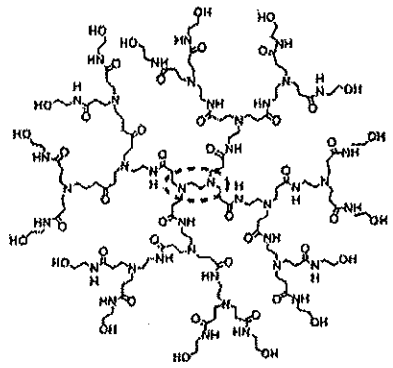


Figure 1. A Generation 2 PAMAM Dendrimer, AuCS-190

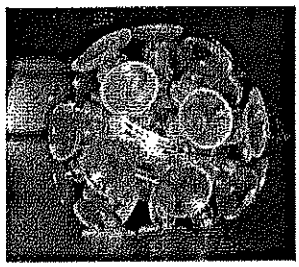


Figure 2. A children's toy is a useful analogy for a dendrimer, think of the suction cups as the surface functional groups

While the utilization of Near-infrared absorbance spectroscopy (NIR-spectroscopy) for OH# determination has been presented in peer review literature of other molecules and with other solvents, dendrimers have not yet been studied by this technique.[3-3b] This is due in part to the relative novelty, high cost, and limited availability of dendrimers. For example, a sample size of PAMAM G6-Dendrimer would cost over $300 for analysis by the titration method and

accurate results are still difficult to obtain. NIR-spectroscopy has the potential to allow for the recovery of the sample or at least to minimize the amount needed for analysis while potentially still providing an accurate determination of the OH# of a dendrimer. The main goal of this project is to investigate the use of NIR-spectroscopy in order to quantify the number of OH functional groups attached to poly(amido amine) or PAMAM dendrimers in a manner that is quick, cost effective and easy to reproduce.[4] The OH number can be calculated theoretically as shown in the equation below for ethylene glycol. By changing the molecular weight of a sample and the number of OH groups per molecule we can arrive at the theoretical OH group number for a sample. The milligrams of KOH consumed in the titration per gram of sample is equivalent to the OH group number.

$$1g\ EG * \frac{1\ mole\ EG}{62.068\ g} * \frac{2\ mole\ OH}{1\ mole\ EG} * \frac{1\ mole\ KOH}{1\ mole\ OH} * \frac{56.1\ g}{1\ mole\ KOH} * \frac{1000\ mg}{1\ g} = 1808\ mg\ KOH$$

Methodology

Sample Preparation

Linear glycol standards of 99% purity as outlined in **Table 1** were purchased from Sigma-Aldrich (see **Figure 3**). The glycols were dissolved in a 20% by weight solution of dimethyl sulfoxide (DMSO) and dried by 3Å molecular sieves. Small samples of approximately 200 µL were then placed into a 1 cm, 200 µL cuvette for analysis by the spectrophotometer. The glycols were PAMAM dendrimers with hydroxyl (-OH) functionality were purchased from commercial sources (Andrews ChemServices, Berrien Springs, MI) as 10 wt% and 20 wt% solutions in methanol or water. Methanol and water contain the –OH group and would interfere with the spectroscopy by overwhelming the absorbance readings. These solvents were removed via solvent evaporation. A stream of nitrogen was used to evaporate the majority of the solvent which was followed by vacuum evaporation with a small amount of heat (no greater than 45 °C) to finish removing the solvent from the solutions. The dendrimer structure will trap small molecules and removal off 100% solvent can be challenging. However, it must be taken into account that the hydroxyl groups in the solvent molecules overwhelm the OH# reading from the dendrimer.[4-5] This is a great challenge since the hydroxyl surface dendrimer is highly soluble in water and alcohol solvents. Therefore, it is helpful to try and remove as much water or methanol from a sample as possible. Small amounts of water can be corrected for by using the water-correction procedure outlined under **Correcting for OH#**.

Dry dendrimers, i.e. dendrimers without a solvent, are generally extremely viscous and must be dissolved in a solvent in order to transfer from a sample container to an analysis chamber or cuvette. Previous research has shown that dimethylsulfoxide (DMSO), a non-protic polar solvent, is the best choice of a solvent and was utilized to dissolve the samples prior to spectroscopy.[5] DMSO is highly polar yet it does not contain –OH groups. Other solvents such as acetone and acetonitrile have been tried but they do not sufficiently dissolve the PAMAM dendrimers. Spectrophotometric grade DMSO was utilized throughout the experiments. Sample solutions consisted of 20% dendrimer sample and 80% DMSO by weight which our previous research has shown to be provide the best signal to noise in the spectroscopic regions under analysis. To decrease the sample size, a quartz submicron cuvette with a 100 µL sample volume and a 1 cm pathlength was utilized for reducing the amount of dendrimer per analysis. The

cuvettes were filled with the solutions and then placed into the Cary 5000 UV-Vis-NIR spectrophotometer.

In order to test absorbance of dendrimers in relation to the OH#, standards must be prepared. The OH# standards, linear glycols, have the OH# determined from the theoretical number since the calibration standards are relatively pure molecules (99% purity and higher). Ethylene glycol, propylene glycol, tri(ethylene) glycol, tetraethylene glycol and polyethylene glycol (MW=1000) were used to generate a calibration curve to benchmark OH# versus absorbance at a variety of wavelengths (see **Table 1**). The calibration curves were analyzed to show the relationship between absorption of NIR light to OH#. The absorptions signals were analyzed to produce the best signal to noise.

Sample Recovery: Because of the large molecular weight and size of PAMAM dendrimers, the dendrimers can be recovered from a mixture of dendrimer and solvent using chromatography or filtration techniques. Preparative HPLC and ultrafiltration equipment are already applied in the manufacturing and preparation of PAMAM dendrimers. Since the dendrimers are not being derivatized, as in the titration method, there is the possibility to recover the costly materials. This makes NIR spectroscopy a valuable alternative to titration for dendrimer materials analysis.

Sample	X	Y	OH#
Ethylene glycol	2	-	1808
Proylene glycol	3	-	1475
Tri(ethylene) glycol	-	3	747
Tetraethylene glycol	-	4	578
Polyethylene glycol	-	~27	112
AuCS-190			270
AuCS-235			628

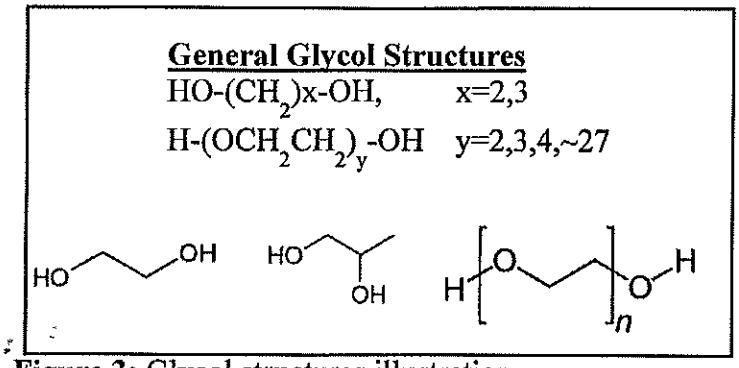


Figure 3: Glycol structures illustration

Table 1: Linear glycols used as standards, structures, and theoretical OH#. Information abou the two dendrimer samples OH# and their theoretical OH# are also provided.

Absorption Spectrum Analysis

A Cary 5000 UV-Vis-NIR Spectrophotometer with a range of 1200-3000 nm was utilized. Infrasil® cuvettes were used due to their limited interference with the beam of light from the spectrophotometer. The baseline sample was established by the use of a cuvette filled with the solvent, DMSO. Experimental samples according to the procedure outlined in the sample preparation section were added to the cuvettes to be scanned. The peak absorbances for the various OH groups not attached to a water molecules were recorded. Mainly the two primary wavelength regions; the R-OH combination band 2000 to 2300 nm and the R-OH first overtone band 1380-1500 nm.[6] Specifically, the absorption signals for 1450, 1580 & 2090 nms show the greatest absorption for the OH signal of a sample. The peak of absorbance of the OH group from water (1940 nm) was also recorded. Care was taken to proceed quickly because of the hygroscopic nature of the samples. The data obtained was then exported to an excel spreadsheet and charted (see **Figure 4**).

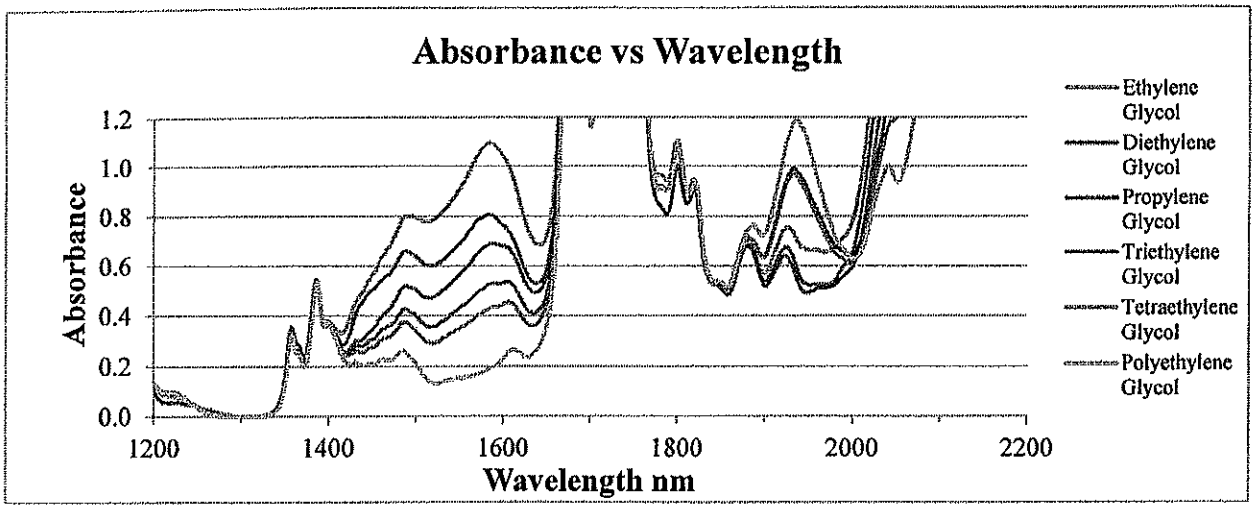


Figure 4: NIR absorbance spectra of 20 wt% glycols in DMSO

Analysis

A variety of linear polyols were utilized as calibration standards in the creation of a calibration curve (see **Figure 5**). The assumption is that linear molecules with many OH groups should provide a usable calibration curve for dendrimers since the analysis should be blind to the architecture of the molecules (linear vs. spherical). A signal to noise ratio analysis was carried out to observe whether the results acquired are significant. If the signal was three times greater than the background noise then the results are taken to be meaningful. Ideal results should provide a calibration curve with a high correlation coefficient value, signifying that the OH# obtained from NIR spectroscopy is accurate to the OH values we expect.

G2-PAMAM-OH and a G2-PAMAM-Tris(OH) that have different theoretical OH# were analyzed. Each one of these dendrimer samples were prepared four times and absorbance spectroscopy performed on each of the samples in order to establish the range of absorbance values possible from one sample. This help in assigning the range of OH# that are possible from this technique. This analysis assists in providing understanding of the versatility and reliability of this method. The theoretical OH#s have been calculated for the PAMAM dendrimers and are shown in **Table 1**.

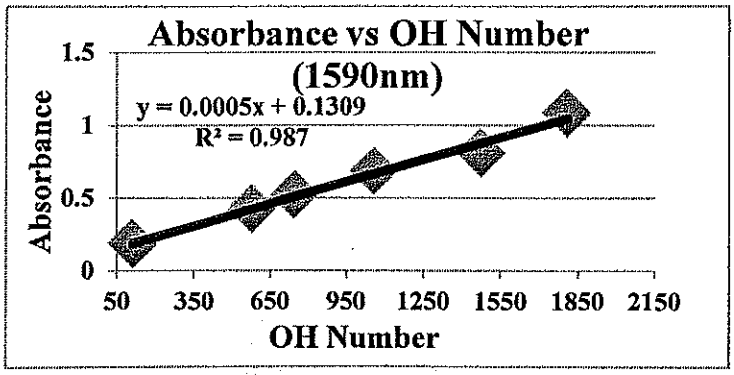


Figure 5: Calibration curve of linear glycols at 1590 nm.

Correcting the OH#

From previous work completed by our research group we have found that it is possible to correct for the OH# of the samples by comparing the peak at 1950 nm (OH groups due to water) to the peak at 1580 nm (the OH groups from the samples and water) (see **Figure 6**). The ratio of 33.5 to 1 exists between the molar absorptivity (slopes) at 1940 nm and 1580 nm (see **Figures 7 & 8**).

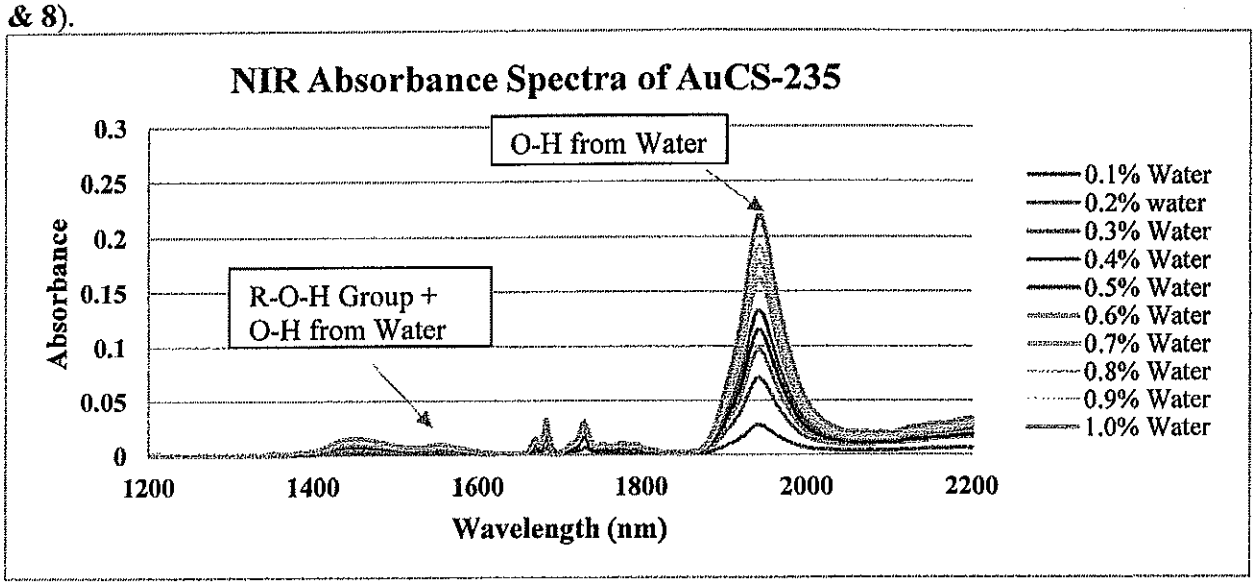
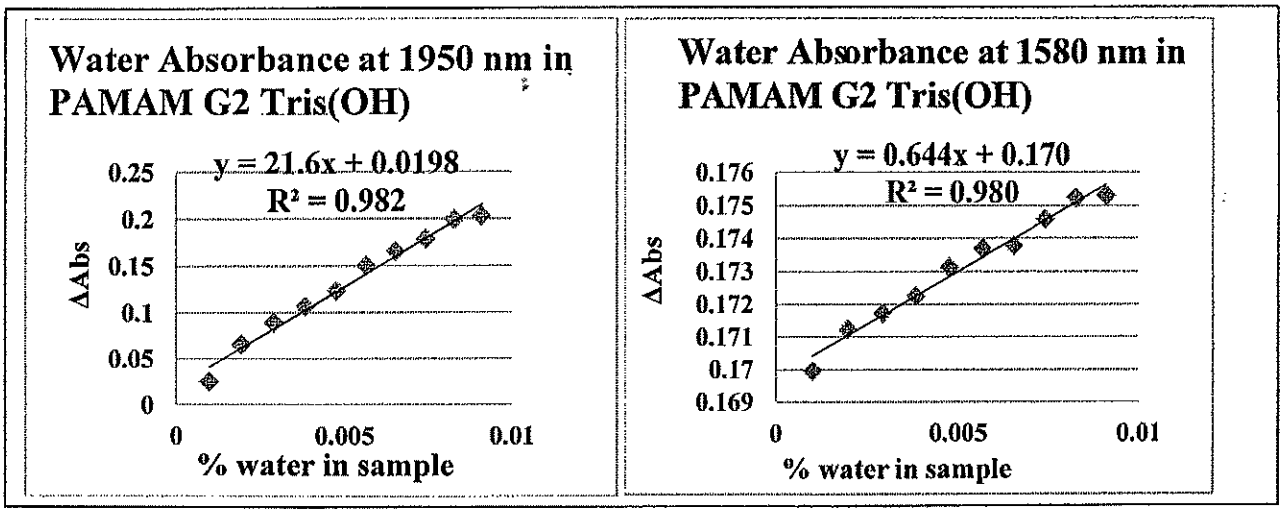


Figure 6: NIR absorbance spectra of linear glycol standards with varying known amounts of water added.



Figures 7 & 8: Calibration curves of samples with known amounts of added H_2O.

The absorbances at 1940 nm were analyzed and peak heights above 0.5533 were considered to have water. Water levels down to 0.1% could be seen with a characteristic peak at 1940 nm. Baselines for water content considered to be "dry" were seen as a relatively flat absorbance spectrum through the 1900 to 1975 nm region but was not at zero absorption. Thus the water weight percent could be determined by the height of this peak at 1940 nm. The first step in the correction process is to determine the difference in absorbance at 1940 nm and then to divide by ratio of the water molar absorptivity at 1940 nm and 1580 nm. Then to subtract this

number from the absorbance reading at 1580 nm and to recalculate the OH# with the new adjusted absorbance at 1580 nm. It must be noted that this data was obtained by the use of a 1 mm cuvette and not the standard 1 cm cuvette utilized in the other experiments.

Results and discussion

Sample	Generation	-OH Groups	Analysis Wavelength	Observed OH #	Adjusted OH #	Theory OH #
AuCS-190	G2	16	1580 nm	451 ± 17	451 ± 17	270
AuCS-190	G2	16	1596 nm	551 ± 13	551 ± 13	270
AuCS-235	G2	48	1580 nm	705 ± 11	647 ± 11	628
AuCS-235	G2	48	1596 nm	809 ± 10	748 ± 10	628

Table 2: Both dendrimer samples were run 4 times to generate these statistical values

Dimethyl sulfoxide still appears to be the best solvent to utilize due to its hydrophilic nature while not containing the OH functional group. 20% by weight solutions provide the least amount of noise with strong signals in the 1580 and 1940 nm regions. This is important because the 1940 nm region is exclusively the OH groups from water whereas the 1580 nm region is composed of the OH groups from the sample and water. This allows for the correction of the OH# by the water correction procedure. The water peak in **Figure 9** for AuCS-235 is quite substantial and this could be due to water being trapped inside the dendrimer's architecture, leftover monomers or impurities.

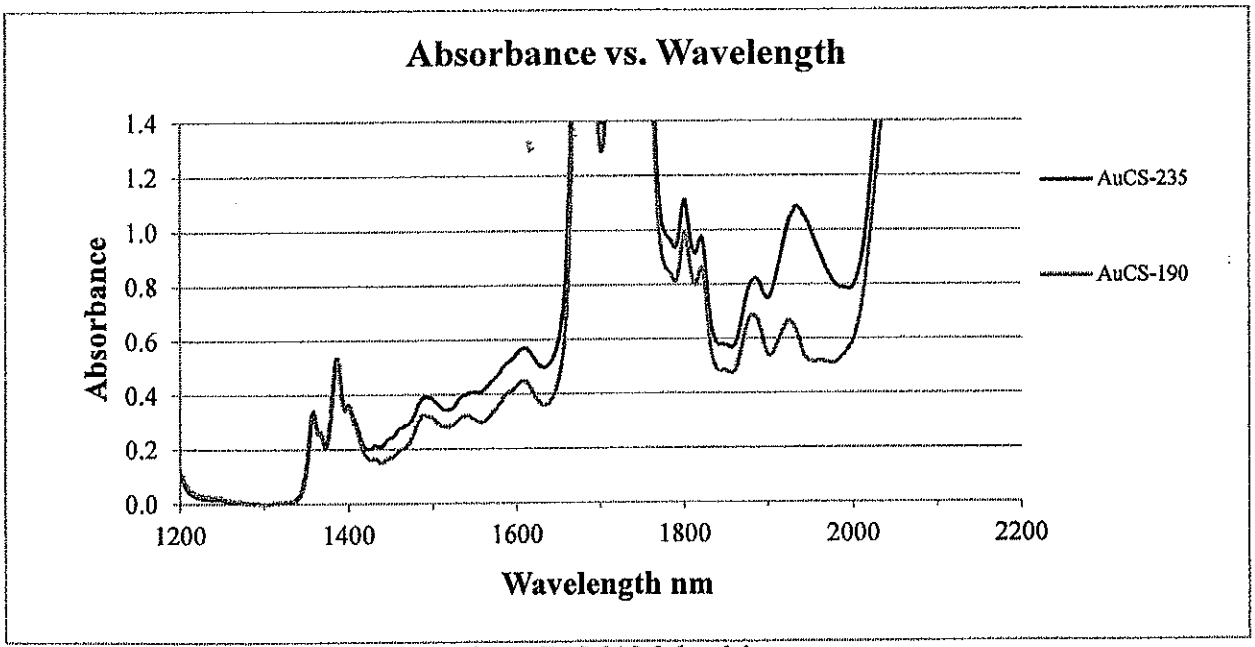


Figure 9: NIR absorbance spectra of two PAMAM dendrimers.

These results, while not accurate in comparison to the theoretical OH# are precise and future work will attempt to discern if impurities exist in the samples and/or if the drying procedure needs refining. The effect of dendritic structure in comparison to linear glycol structures will also be investigated.

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