

## Electronic Supporting Information

# Self-Diffusion of Water and Poly(Amidoamine) Dendrimers in Dilute Aqueous Solutions

István Bányai, Mónika Kéri, Zoltán Nagy, Márta Berka, and Lajos P. Balogh

- 1. Analytical methods and typical results (Dendrimer characterization):**
- 2. Determination of p*K*s for PAMAM\_E5.X dendrimers**
- 3. Assignment of protons for PAMAM\_E5.X**
- 4. Diffusion experiments and analysis:**
- 5. Concentrations and volume fractions of the dendrimer solutions used**

## 1. Analytical methods and typical results (Dendrimer characterization)

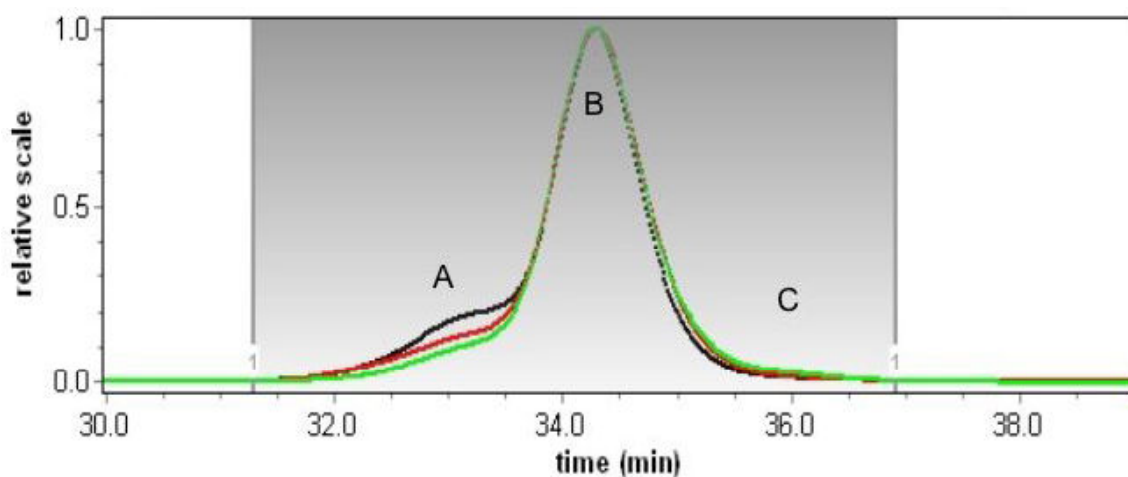
Table I: Summary of characterization data of dendrimers used (average values).

Dendrimer	Mn	Mw	Mw/Mn	Number of termini measured
PAMAM_E5.NH2	29260	30720	1.050	119
PAMAM_E5.SAH	38960	40710	1.045	117

### 1.1. PAMAM\_E5.NH<sub>2</sub> Dendrimer (E5.NH<sub>2</sub>, lot# 1205-06)

#### 1.1.1 Average molecular mass

Size Exclusion Chromatography (SEC) has been used to determine the average molecular mass of the PAMAM\_E5.NH<sub>2</sub> dendrimer. Measurements were performed using an Alliance Waters 2690 separation module equipped with an ultraviolet-visible (UVvis) detector (Waters Corp, Milford MA, USA), a Wyatt Dawn laser (690 nm) photometer and an Optilab interferometric refractometer, operating at 685 nm (Wyatt Technology Corporation, Santa Barbara, CA, USA). Phosphate buffer (0.05 M, pH 2.5) with 0.025% sodium azide was used as the mobile phase. The flow rate was maintained at 0.6 mL/min. For separation, three Waters columns were used: Ultrahydrogel 500 (7.8x300mm), Ultrahydrogel 250 (7.8x300mm) and Ultrahydrogel 120 (7.8x300mm). Sample concentration was kept at 2 mg/mL and 100  $\mu$ L was injected. Data were elaborated using Astra, Empower and PeakFit software packages (Wyatt Technology Corporation, Santa Barbara, CA, USA; Waters Corp, Milford, MA, USA and San Jose, CA, USA).

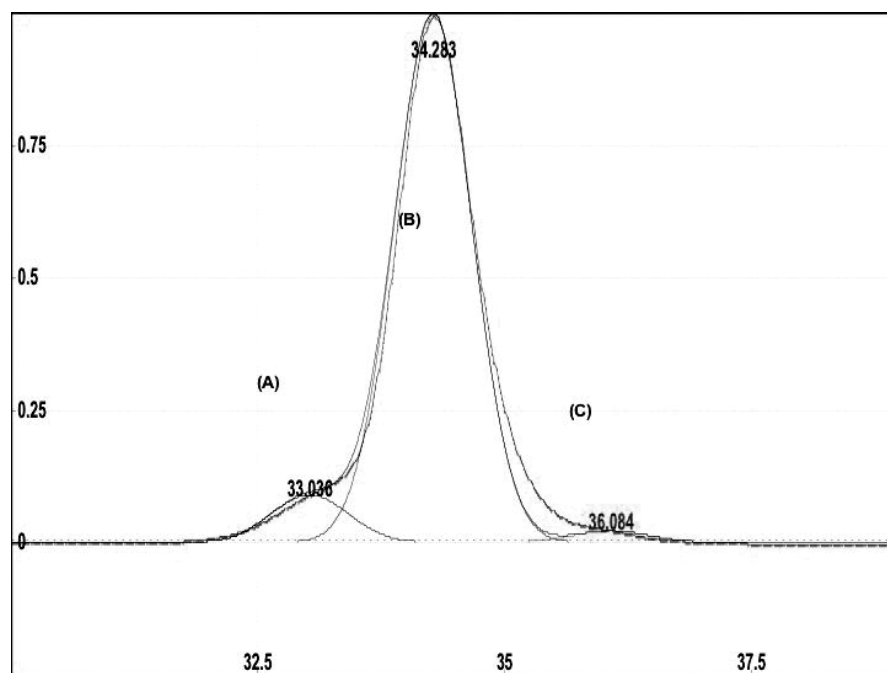


**Figure 1.1:** Elution curves of an SEC run of PAMAM\_E5.NH<sub>2</sub>, lot# 1205-06-E5.0-LD). The grey area indicates the areas considered as the total material. Black line: MALLS 90° detector, red line: UV absorbance @254 nm, green line: RI detector

A summary of analytical data are presented in Table I.

Molar Mass Moments (g/mol): Mn=29260, Mw=30720, Polydispersity Mw/Mn=1.050

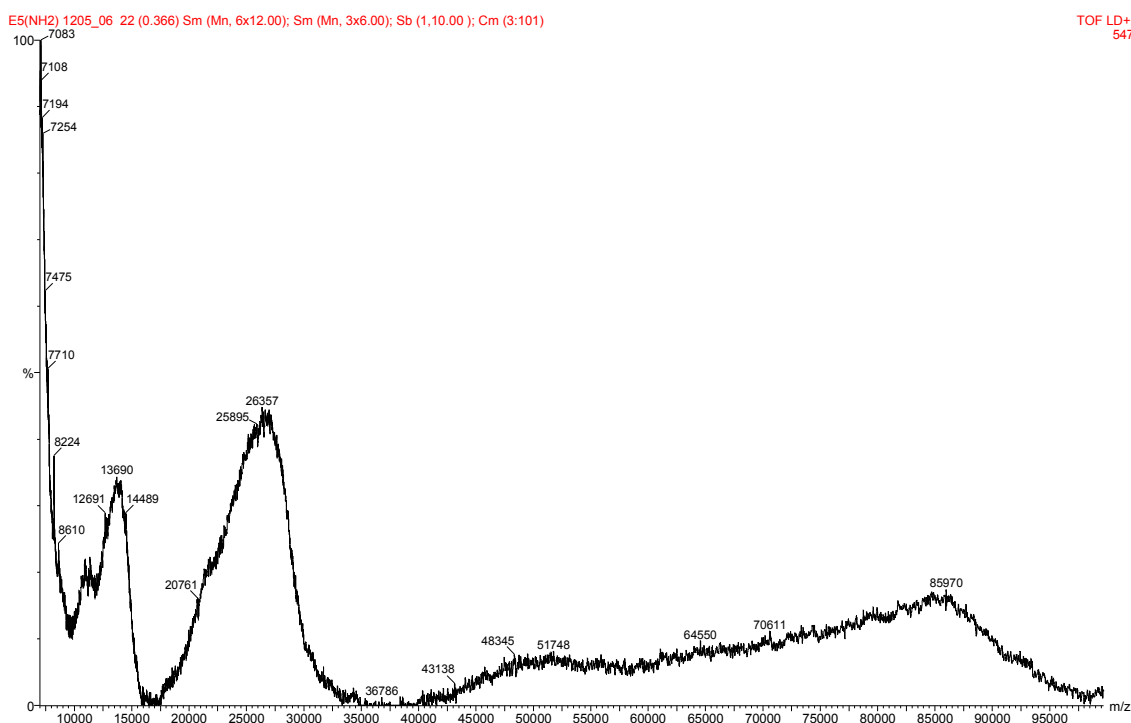
Due to generational heterogeneity originating from the PAMAM synthesis, in addition to the main mass fraction (here E5.NH<sub>2</sub>), every practical PAMAM material contains covalent dimers of the same generation (here (E5.NH<sub>2</sub>)<sub>2</sub>), and a trailing generation



**Figure 1.2.** According to Peakfit analysis, this PAMAM<sub>5</sub>.NH<sub>2</sub> dendrimer contains 8.1% dimers (E<sub>5</sub>.NH<sub>2</sub>)<sub>2</sub> (A), 89.6% E<sub>5</sub>.NH<sub>2</sub> generation five dendrimers (B) and 2.3% of a trailing generation (E<sub>4</sub>.NH<sub>2</sub>) dendrimers.

**Matrix Associated Laser Desorption (MALDI-TOF):**

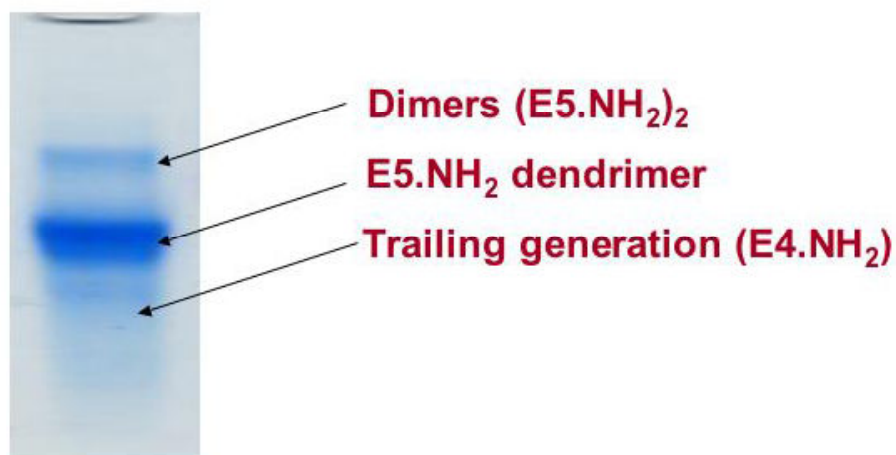
MALDI-TOF spectra were obtained, using a Micromass ToFSpec-2E mass spectrometer in reflection mode. 2,5-Dihydroxybenzoic acid (DHB) was used as a matrix ( $c = 10$  mg/mL). First, 0.5 mL of matrix was spotted on target plate and evaporated. Then 0.5 mL of the dendrimer (1.5 mg/mL) was spotted over the matrix. Matrix and dendrimer were dissolved in 75% acetonitrile:25% water solution containing 0.1% TFA (trifluoroacetic acid). For each spectrum 100 shots and 50% of the maximum laser power was applied.



**Figure 1.3.** Peak values (g/mol): 13690, 26357, and 51748 respectively

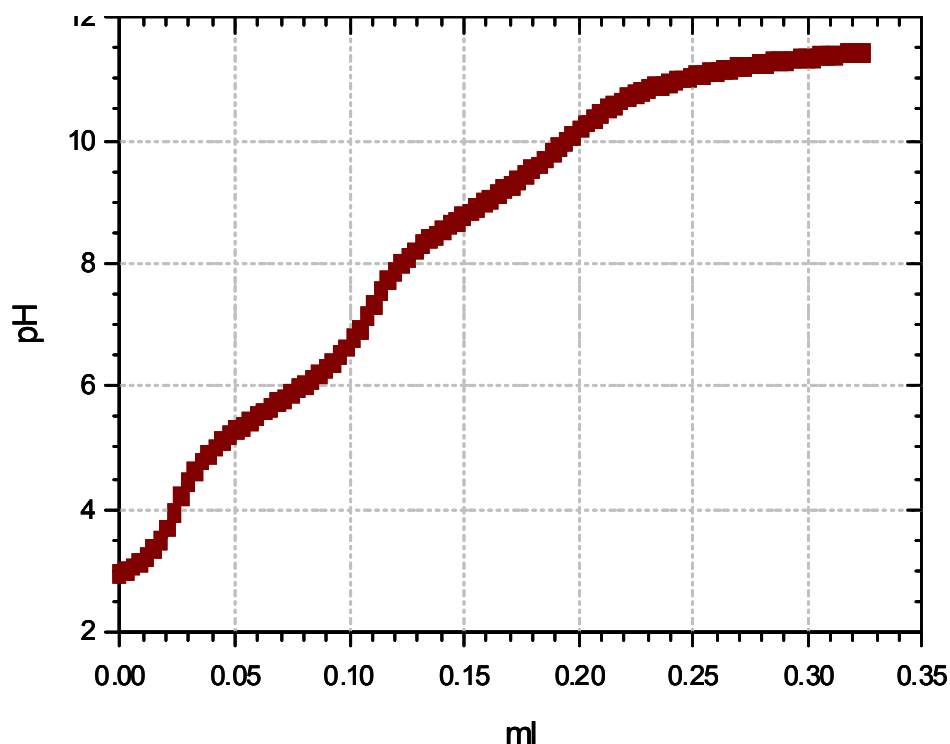
### **Polyacrylamide Gel Electrophoresis (PAGE)**

Generational heterogeneity was qualitatively confirmed by PAGE. PAGE was performed using a vertical electrophoresis system, precast 4-20% gradient express gels and Tris-glycine (TG) native (pH = 8.3). Separations were done for 1 h at 200 V and 100 mA. Developed gels were stained with 0.025% Comassie Blue R-250 in 40% methanol and 7% acetic acid aqueous solution overnight. The gels were destained in 7% (v/v) acetic acid and 5% (v/v) methanol in water.



## 2. Determination of pKs for PAMAM\_E5.X dendrimers

### 2.1. PAMAM\_E5.NH<sub>2</sub>



**Figure 2.1.** Titration curve of PAMAM\_E5.NH<sub>2</sub>. The calculated curve is covered by the experimental points.  $C$  (dendrimer) = 1mg/cm<sup>3</sup>, under Ar.

The experiments were performed with addition of HCl in excess and by titration of KOH. The ionic strength was 0.2 mol dm<sup>-3</sup> KCl. The parameter fitting was performed by means of Hyperquad software. A simple solution equilibrium model was used.

Results:

**pK<sub>N(T)</sub> = 5,72(2), pK<sub>N(P)</sub> = 8,90(2)**

**119,0 pcs of terciar N-s, 122,6 pcs of primerN-s per molecule dendrimer.**

### 2.2. Protonation constants of different generation PAMAM-E5.SAH dendrimers using different models for determination the protonation constants. (FITEQL)

The three tables show the protonation constants of E3-5\_PAMAM.SAH (generation 3-5) dendrimers evaluated at different methods from same titration curves. In the first Table we used fixed number of groups (as ideal dendrimer) and no surface complexation model was applied. ( $W$  is weighted sum of squares of the difference, divided by degrees of freedom, at the best fit. Fit is excellent if  $W < 20$ )

In the second Table we allowed to refine the number of functional groups but no surface complexation model was used. (See red numbers)

In the third Table we used a constant capacity surface complexation model and supposed that the number of functional groups corresponds to ideal dendrimers.

It can be seen that the last two models describe the titration curves equally well therefore both model can be used. It is also a matter of discussion how the number of TN (tertiary nitrogens) and COOH groups affect the number of each other.

group	Dendr.	COOH	TN	lg K <sub>COOH</sub>	lg K <sub>TN</sub>	W
62	e3	32	30	3.87	5.97	61
126	e4	64	62	4.14	6.37	36
254	e5	128	126	4.32	6.64	36

group	Dendr.	COOH	TN	lg K <sub>COOH</sub>	lg K <sub>TN</sub>	W
<b>52</b>	e3	33	<b>22</b>	4.45	6.46	24
<b>117</b>	e4	65	<b>52</b>	4.52	6.63	17
<b>225</b>	e5	117	<b>108</b>	4.60	6.69	9

group	Dendr.	COOH	TN	lg K <sub>COOH</sub>	lg K <sub>TN</sub>	W
62	e3	32	30	<b>4.55</b>	<b>5.12</b>	29
126	e4	64	62	<b>4.46</b>	<b>6.02</b>	19
254	e5	128	126	<b>4.61</b>	<b>6.45</b>	4

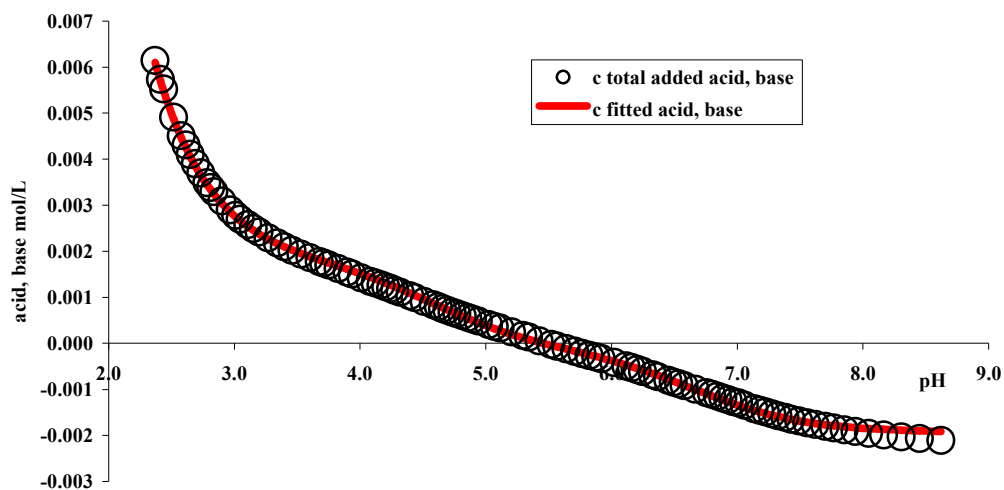
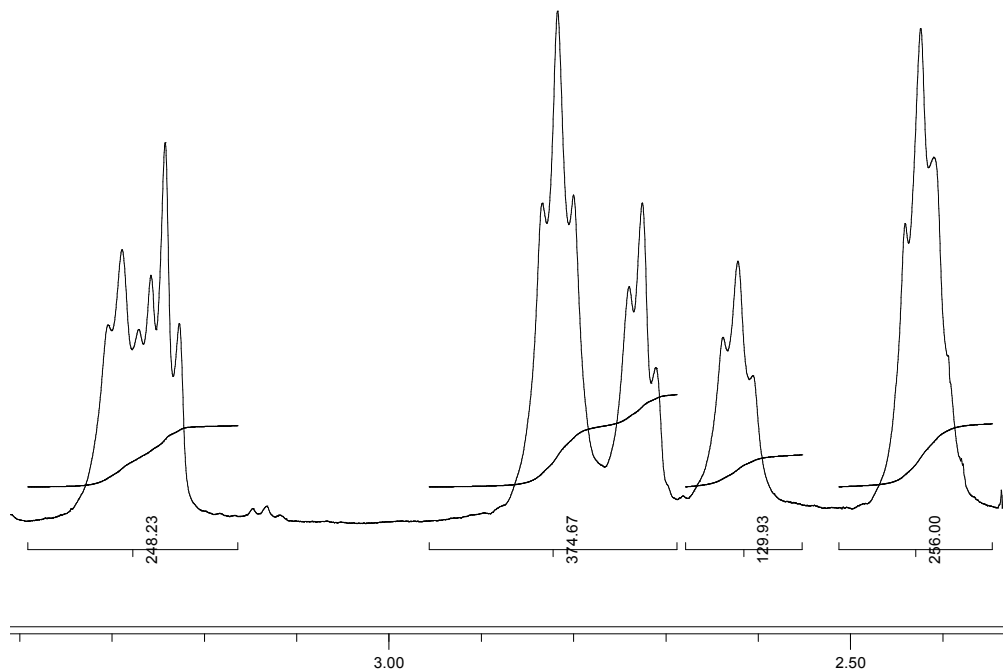


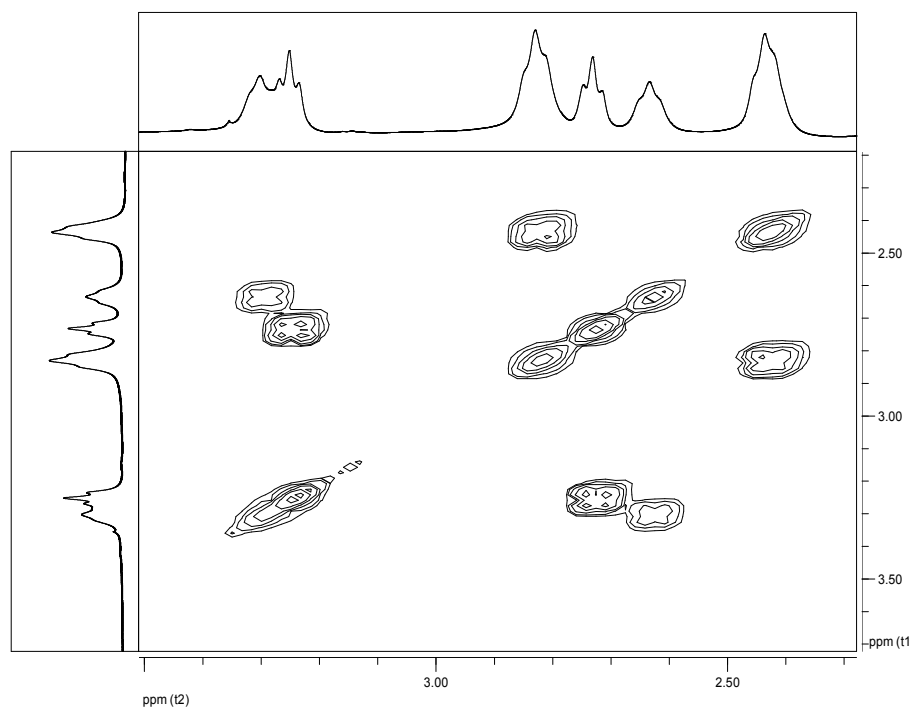
Figure 2.2. Measured and calculated total acid content by FITEQL for E5. SAH

### 3. Assignment of protons for PAMAM\_E5.X

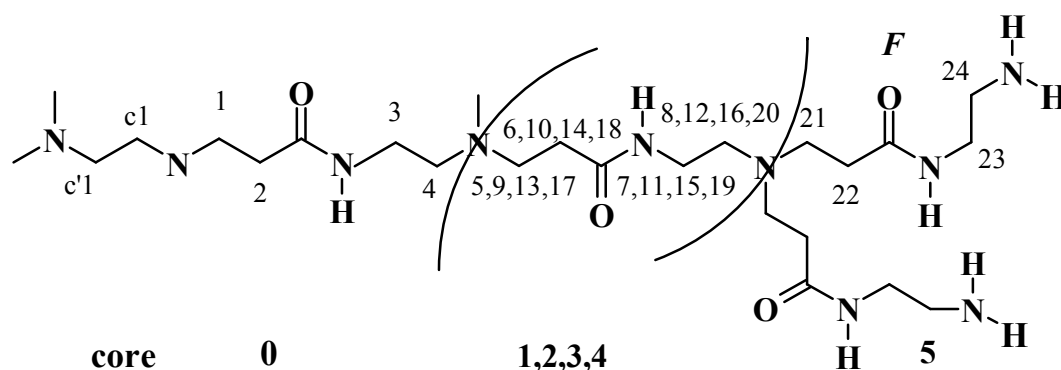
#### 3.1. PAMAM\_E5.NH<sub>2</sub>



**Figure 3.1.** <sup>1</sup>H-NMR spectra of PAMAM\_E5.NH<sub>2</sub> at self pH.



**Figure 3.2.** <sup>1</sup>H-NMR and <sup>1</sup>H-<sup>1</sup>H COSY spectra of PAMAM\_E5.NH<sub>2</sub> at self pH



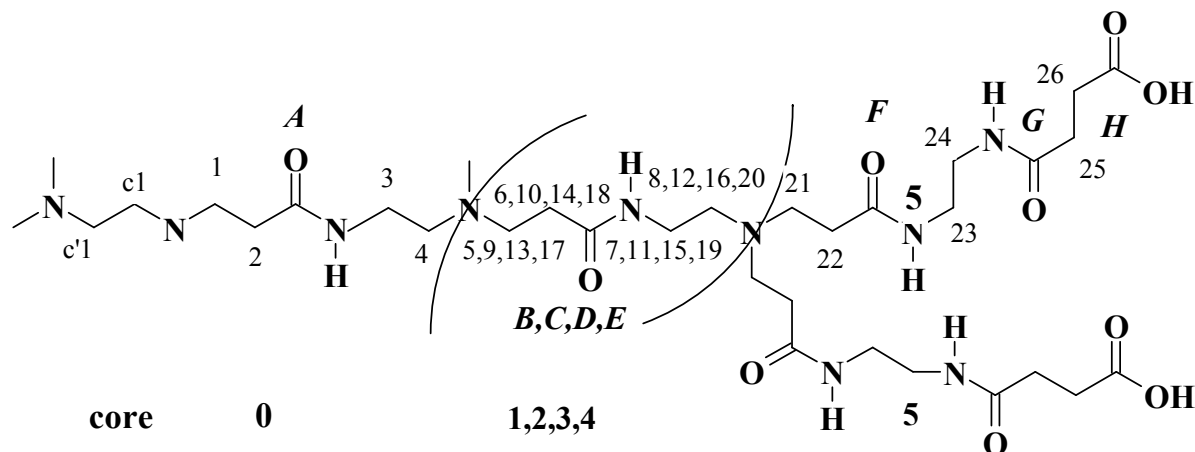
From the integrals  $\Sigma$  2-22 and  $\Sigma$  1-21 are easily assigned at 2.48 and 2.87 ppm respectively. It is possible to choose which is which from the chemical shifts and from the HSQC experiment. 24 (2.77 ppm) and 23 (3.29 ppm) and  $\Sigma$  4-20 (2.67 ppm) and  $\Sigma$  3-19 (3.34 ppm) can be assigned from COSY and on the basis of integrals. The assignment can be found in many literatures.

(For example: X. Shi, I. Bányaı, M.T.Islam, Wojciech Lesniak, Denzel D. Davis, James R. Baker Jr., Lajos P. Balogh: **Generational, skeletal and substitutional diversities in generation one poly(amidoamine) dendrimers.**

*Polymer* 3022 46 2005)

### 3.2. Assignment of $^1\text{H}$ NMR signals of PAMAM\_E5.SAH

The formula used:



The meaning of the letters and numbers are as follows:

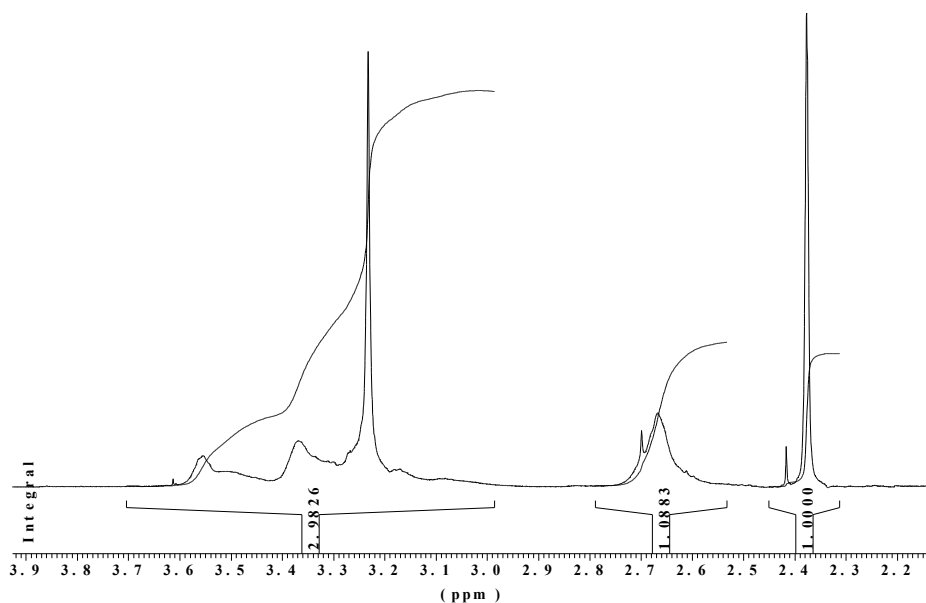
c1 is the core carbon (and protons)

1-25 are the  $\text{CH}_2$  carbons and the protons on them

A-H are the carbonyls

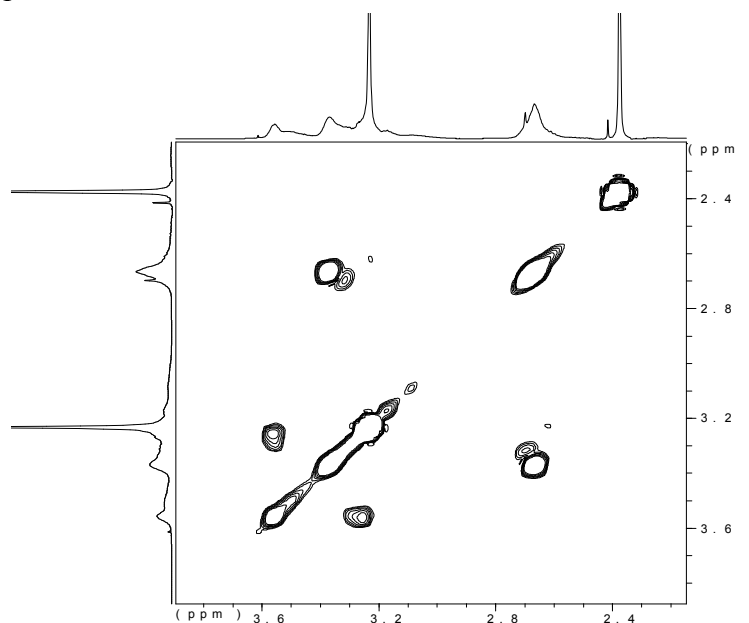
1-5 are number of generations





**Figure 3.3.** 500 MHz NMR of PAMAM\_E5.SAH at self pH. (pD= 5)

The assignment is not trivial however it can be done. From Figure 1 we can easily identify two singlets as 25, 26 (2.38 ppm) and 23, 24 (3.22 ppm) protons on the basis of their integrated intensity. The equivalence of 25, 26 is not trivial but in fact when the COO<sup>-</sup> group is not protonated it is possible. The rest of the peaks: at 2.66 ppm is  $\Sigma$  (2-22) (all the similar protons). From COSY (Figure 4.4.) we could get the  $\Sigma$  (1-21) at 3.35 ppm (it is actually not a single peak but a group of peaks because of the slightly different chemical environment from 1-21) on the basis of the cross peaks indicated scalar coupling. The rest two peaks (groups of peaks) are  $\Sigma$  (3-19) and  $\Sigma$  (4-20) are identified on the basis of their integrals and COSY cross peaks.



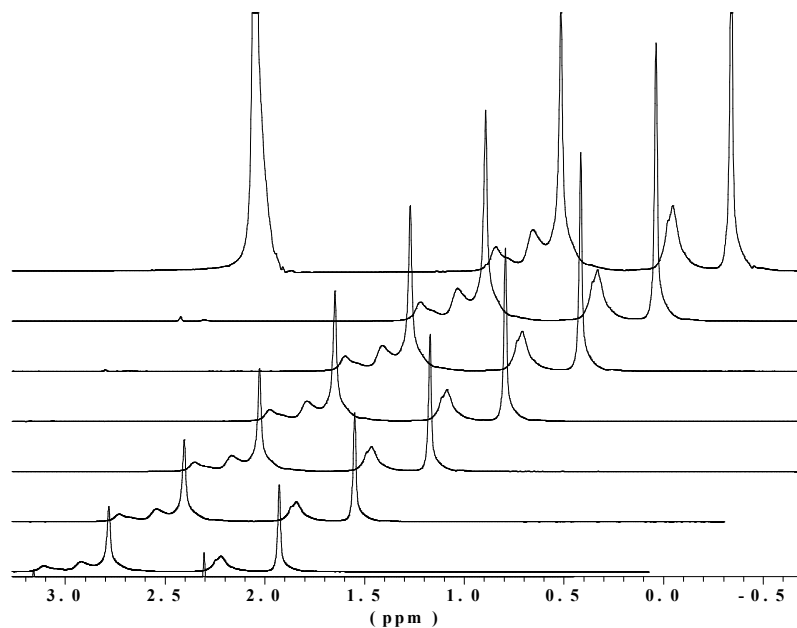
**Figure 3.4.** <sup>1</sup>H 2D COSY of PAMAM\_E5.SAH at self pH (pD= 5)

#### 4. Diffusion experiments and analysis:

Diffusion experiments were performed according to the usual way. We studied the attenuation of the signals as a function of square of applied pulse field gradient pulse. The decrease of integrated intensity corresponds to the following equation:

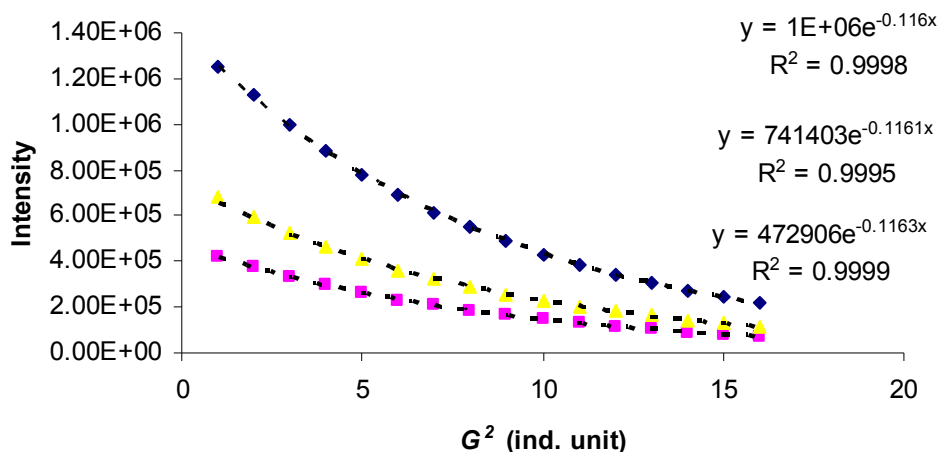
$$I = I_0 / 2e^{-\left(\frac{2\tau}{T_2} + \frac{T_M}{T_1}\right)} e^{-\left(\gamma^2 \delta^2 \left(\Delta - \frac{\delta}{3}\right) D G^2\right)}$$

where the parameters are known from, e.g. Johnson Jr, C.S. *Progr. Magn. Reson. Spect* **34** 203 (1999). Figure 4.5. shows a typical series of NMR spectra as a function of  $G^2$  with all the other parameters constant.



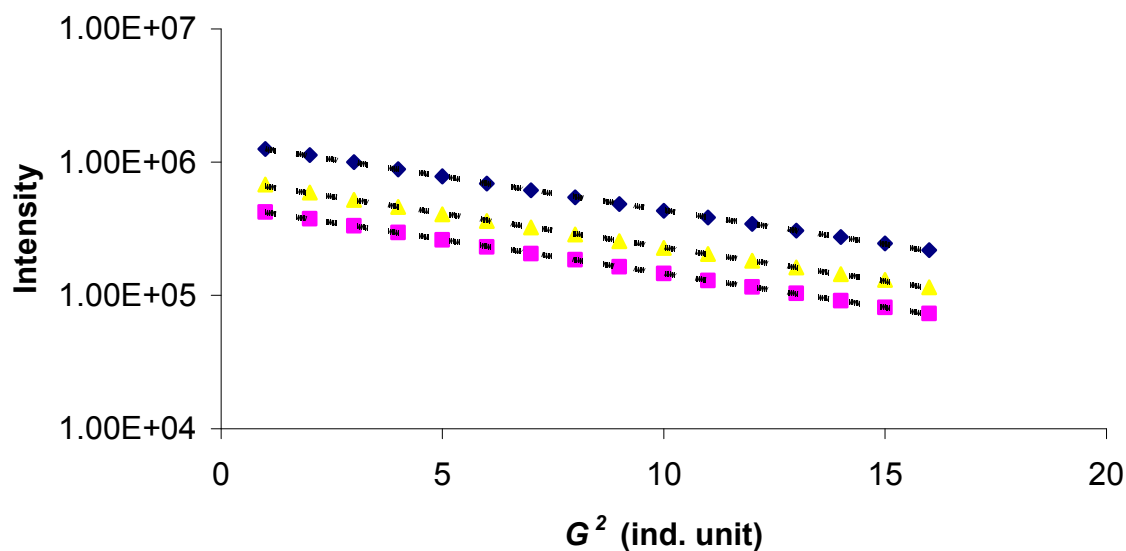
**Figure 4.1**  $^1\text{H}$  NMR spectra of PAMAM\_E5.SAH as function of the strength of square gradient pulse. 7 curves of 16 are shown for the sake of clarity.

Typically 16 curves were recorded and according to Figure 5.1. Within an experiment  $\Delta$  and  $\delta$  were constants while the gradient ramp changed. The integration was made in three parts. Exponential functions were fitted on the integrated intensity vs,  $G^2$  data as it is shown in Figure 5.2. For one cone concentration 3-5 different  $\Delta$  and  $\delta$  were values selected. Those were selected in which the decay was between 80 and 95 %. No significant tendency of the exponents were found at different  $\Delta$  and  $\delta$  values only the statistics of parameter fitting were better.



**Figure 4.2.** The change of integrated intensity of three parts as function of square of gradient pulse power.  $c = 0.41$  mM, pH=5.4 The fitting results are reported in top right corner.

The  $D$  was calculated from the exponential parameter according to equation above. The absolute values of diffusion constants were determined using the results of PGSE study of  $D_2O$  sample and the literature value of the diffusion coefficient. (Holz, M.; Heil, S. R.; Sacco, A. *Phys. Chem. Chem. Phys.* **2**, 4740-4742 (2000)).



**Figure 4.3.** Linearized curves, semi log plots of Figure 4.6

## 5. Concentrations and volume fractions of the dendrimer solutions used

### 5.A: PAMAM\_E5.NH2

C experimental (g/kg)	C mol/kg	$D_{\text{dend}}$ ( $\text{m}^2/\text{s}$ )	Size m	
		0	6.67E-11	<b>3.040E-09</b>
46.10	0.001500651	4.9930E-11	<b>4.068E-09</b>	
38.33	0.001247783	5.2621E-11	<b>3.860E-09</b>	
32.82	0.001068400	5.5516E-11	<b>3.658E-09</b>	
24.35	0.000792643	5.7934E-11	<b>3.506E-09</b>	
19.22	0.000625740	6.0523E-11	<b>3.356E-09</b>	
11.30	0.000367839	6.2021E-11	<b>3.275E-09</b>	

c (g/kg)	fi_hidr	fi(18Å rule)	fi(Hardsph)
0.0000	0.0000	0.0000	0.0000
11.3000	0.0285	0.0090	0.0174
19.2227	0.0479	0.0153	0.0296
24.3500	0.0602	0.0194	0.0373
32.8209	0.0802	0.0262	0.0501
38.3319	0.0929	0.0306	0.0583
46.1000	0.1104	0.0369	0.0698

### 5.B: PAMAM\_E5.SAH

C exp (g/kg)	C mol/kg	$D_{\text{dend}}$ ( $\text{m}^2/\text{s}$ )	Size m
45.30744	0.001089	4.3008E-11	<b>4.722E-09</b>
36.55352	0.000879	4.6848E-11	<b>4.335E-09</b>
27.77778	0.000668	5.0688E-11	<b>4.007E-09</b>
18.62941	0.000448	5.2032E-11	<b>3.903E-09</b>
9.402283	0.000226	5.7408E-11	<b>3.538E-09</b>
4.723347	0.000114	5.7408E-11	<b>3.538E-09</b>
0	0	5.9800E-11	<b>3.396E-09</b>

c (g/kg)	fi (hydr)	fi (18 Å rule)
45.30744	0.1180374	0.041070557
36.55352	0.0966538	0.033106865
27.77778	0.074566	0.025137001
18.62941	0.0508137	0.01684328
9.402283	0.0260692	0.008493167
4.723347	0.0132067	0.004264694
0	0	0