

Novel Functionalised Polyamidoamine (PAMAM)

Dendrimers: Synthesis and Physical Properties.

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Abstract:

We report the synthesis and characterisation of a series of polyamidoamine (PAMAM) dendrimers, functionalised at the amine end group with *p*-nitrobenzyl, 2-naphthyl and tricyanoquinodimethyl (TCQ) moieties. The fluorescence spectral characteristics and electric field induced second harmonic generation behaviour were also investigated.

Introduction

As a class of new materials, dendrimers or ‘starburst’ polymers have generated great interest throughout the scientific community. Despite synthetic difficulties^{1,2}, a wide range of these new materials have been produced and characterised^{3, 4, 5}. However the study of their properties is still in its infancy (relative to classical polymers) and there remains large gaps in our knowledge which must be filled if the potential uses of these materials are to be realised.

Our interest in dendrimers stems from their mesoscopic physical scale and well defined overall size⁶. Control of the size and geometry of molecular structures at

dimensions in the order of 100 nm may offer an opportunity to observe new physical phenomena specifically related to the boundaries imposed by the molecular structure. Restriction to a well defined molecular region of either discrete charge carriers or of extended electronic excitations may result in novel phenomena not observable at other size scales. Such effects would require some degree of correlation between sub-structures in the molecule such as interaction between closely spaced surface functional groups^{7, 8}.

Our work has yielded a range of functionalised polyamidoamine (PAMAM) dendrimers. These contain either dipolar, nonlinear optical or fluorescent groups. Initial evaluation of these systems has been undertaken including attempts to characterise the molecules using fluorescence spectroscopy and Electric Field Induced Second Harmonic generation (EFISH).

Experimental Section

PAMAM dendrimer and model compound synthesis. PAMAM dendrimers derived from an ammonia core⁹ (such compounds will be referred to as ‘**AG_x**’ in the text, where **A** denotes the ammonia core, **G_x**, the generation number and **x** = 1-7) and the model compounds 2-naphthylamide (NAM) and 1,2-di(amido-2-naphthalene)ethane¹⁰ (DINAM) were prepared by literature methods. PAMAM dendrimers derived from an ethylenediamine core (such compounds will be referred to as ‘**EG_x**’ in the text, where **E** denotes the ethylenediamine core) were purchased from Aldrich. All solvents used were Aldrich HPLC grade and all chemicals were used as received from Aldrich, except for tetracyanoquinodimethane (TCNQ), which was purchased from Lancaster.

General procedure for synthesis of 2-naphthyl-functionalised dendrimers, Series I¹⁰. A water/acetonitrile solution of 2-naphthoyl chloride was added dropwise to a stirred solution of aqueous sodium hydroxide and PAMAM dendrimer. The resulting mixture was stirred for 48 hours under nitrogen, then washed thoroughly with ether to remove excess 2-naphthoyl chloride. The water was removed by rotary evaporation and the remaining white solid was crystallised from water/methanol. Subsequent recrystallisations from methanol gave a white powder in yields that varied from 30 - 40%. Characterisation was achieved by proton and carbon NMR, infra-red and UV/visible spectroscopy. Data for compound **IAG1**; ir (KBr disc) ν_{CO} 1633, 1538 cm^{-1} ; UV/vis (methanol) λ 235, 280, 329 nm; ^1H NMR (d_6 DMSO, ppm) δ 1.31 (triplet, broad, 2H), δ 1.78 (triplet, broad, 2H), δ 2.42, 2.48 (multiplet, broad, partially obscured DMSO, 4H), δ 6.69 (quintet, 2H), δ 7.08 (doublet, 4H), δ 7.53 (singlet, broad, 1H), δ 7.60 (singlet, 1H), δ 7.88 (triplet, broad, 1H). ^{13}C data for compound **IEG5** (d_6 DMSO) δ 30.71, δ 33.31 (broad), δ 36.91, δ 37.00, δ 37.21, δ 49.59 (broad), δ 52.16 (broad), δ 124.18, δ 126.09, δ 126.65, δ 127.01, δ 127.52, δ 127.79, δ 128.80, δ 131.77, δ 132.11, δ 134.08, δ 166.49, δ 171.34, δ 171.83, δ 172.22. GPC analysis was also carried out on this series.

General procedure for synthesis of *p*-nitrobenzyl-functionalised dendrimers, Series II¹¹. A DMF solution of PAMAM dendrimer was added dropwise to a stirred mixture of 4-nitrobenzylchloride and sodium hydrogen carbonate in DMF under nitrogen. The reaction was warmed at ~ 50 °C for 2 hours and then stirred at room temperature for 48 hours. Sodium hydrogen carbonate was removed by filtration and the DMF was removed by rotary evaporation, yielding a red oil which was stirred vigorously with a large volume of ether overnight to remove excess 4-

nitrobenzylchloride and yielded the crude product as an orange powder. This was separated by filtration and subsequently dissolved in dichloromethane, filtered and crystallised from acetone/ether (higher generations were precipitated by the addition of a DMF solution to a large volume of acetone), yielding a yellow/orange powder in yields of 30 - 43%. Product characterisation was achieved using infra-red, UV/visible and proton and carbon NMR spectroscopy. This series of functionalised dendrimers were also studied by GPC. Data for compound **IIAG1**; ir (KBr disc) ν_{CO} 1642, 1517 cm^{-1} ; ν_{NO_2} 1558, 1344 cm^{-1} ; UV/vis (DMF) λ_{max} 277 nm; ^1H NMR (CD_2Cl_2 ppm) δ 2.23 (triplet, 2H), δ 2.50 (triplet, 2H), δ 2.65 (triplet, 2H), δ 3.22 (quartet, 2H), δ 3.63 (singlet, 4H), δ 6.51 (triplet, 1H), δ 7.51 (doublet, 4H), δ 8.11 (doublet, 4H); ^{13}C NMR (CD_2Cl_2 ppm) δ 35.68, δ 38.72, δ 51.23, δ 55.38, δ 59.71, δ 125.40, δ 131.33, δ 148.87 δ 149.06, δ 173.92.

General procedure for synthesis of TCNQ-functionalised dendrimers, Series III¹². A solution of PAMAM dendrimer in DMF (dimethylformamide) was added to a warmed, stirring solution of TCNQ (tetracyanoquinodimethane) under nitrogen. After stirring for 48 hours, the DMF was removed by rotary evaporation and the resulting solid was dissolved in aqueous sodium hydroxide, filtered and then acidified with dilute hydrochloric acid which brought about precipitation of a black/purple solid. This was collected by filtration, washed with water and dried under vacuum. The products were characterised by proton NMR, infra-red and UV/visible spectroscopy. Data for compound **IIIAG2**; ir (KBr disc) ν_{CO} 1654, 1576, ν_{CN} 2172, 2123; UV/vis (DMF) λ 368, 446 nm.

Spectroscopic Measurements. ^1H and ^{13}C NMR spectra were obtained using a Bruker AC250 or a Varian VXR-200 spectrometer. Infrared spectra were measured

by a Perkin Elmer 1600 series FT-IR spectrometer. UV/vis spectra were recorded using a Perkin Elmer Lambda 19 spectrophotometer. Fluorescence spectra were obtained by use of a Perkin Elmer LS50B Luminescence spectrometer. GPC was performed on a PL gel 5 μm mixed C polystyrene, 300 x 7.5 mm column with NMP (N-methylpyrrolidinone) and lithium chloride (~ 0.1% by weight) as the eluent. The operating temperature was 80 $^{\circ}\text{C}$, the pressure was 5 MPa, the flow rate 1 mL min^{-1} and the sample injection coil size was 20 μL . A Pye Unicam 4011 pump with a 4030 controller, a 4810 integrator and a 4020 uv detector were used.

Results and Discussion

Initially, both functionalisation of 'whole' generation (NH_2 terminated) and 'half' generation (CO_2^- terminated) PAMAM dendrimers was attempted, of which the amine chemistry was found to be more immediately successful. Scheme 1 illustrates the three most successful reactions investigated and discussed herein. Two types of PAMAM dendrimer; one type possessing an ammonia (NH_3) core and the other having an EDA (ethylenediamine, $\text{H}_2\text{NCH}_2\text{CH}_2\text{NH}_2$) core were investigated. In all three reaction series studied, a large excess of functionalisation reagents was used (typically two molar equivalents per dendrimer reactive site) and extended reaction times were employed to ensure complete reaction of the dendrimer end groups.

Ir data obtained from the compounds of series **I** (**IAG1**, **IAG2**, **IEG1**, **IEG2**, **IEG3**, **IEG4**, **IEG5** and **IEG7**), showed the absence of the peak at 1750 cm^{-1} , due to the carbonyl stretch of the starting 2-naphthoylchloride compound and strong peaks due to the carbonyl stretch of the amide groups, which shifted to higher wavenumber in higher generations, indicative of increased hydrogen bonding within the structure. In higher generations (compounds **IEG4**, **IEG5** and **IEG7**) a strong, unidentified peak at 1399 cm^{-1} was observed. Structured peaks attributed to C-H and N-H stretching in

the 3500 - 2500 cm⁻¹ region were observed in the lower generations, which tended to broaden into two single peaks in higher generations. ¹HNMR showed the expected peaks due to the CH₂ groups of the dendrimer (the CH₂ next to the newly formed amide bond was shifted to higher ppm), the aromatic naphthyl unit and discrete amide resonances. At higher generations, however the ¹HNMR became increasingly broad; the only useful information that could be extracted was the relative proportions of alkyl:aromatic protons from the integration. Even this crude analysis was complicated by the presence of the peak due to the incompletely deuterated solvent and also to residual methanol contained within the sample, which could not be removed even by prolonged drying procedures (over one week in a vacuum desiccator). Greater reliance was therefore placed on ¹³CNMR. In the case of **IEG5** an additional carbon resonance was observed at δ 172.22 ppm, indicative of a dislocation in the dendrimer structure^{1,2}. Examination of UV/vis data shown in Table 1, of the compounds of series **I**, showed the approximate expected increase of the extinction coefficient on moving to higher generations. This indicated that the dendrimer end groups were fully functionalised. In GPC traces obtained from this series, sharp peaks of low polydispersity were observed for the lower generations. Samples **IEG3** and **IEG4** displayed an additional peak. Disagreement between found and expected molar masses (Table 2), may be due to the polyvinylpyridine standards used which are a poor model at lower molar masses. Initial work, showing unexpected high molecular mass peaks in the GPC trace, suggested that aggregation of dendrimer molecules was occurring even at the low working concentrations of the experiment. The addition of lithium chloride (~ 0.1% by weight) to NMP solvent prevented this.

Of series **I**, compound **IEG7** was too insoluble to carry out NMR or GPC analysis and only its and poor quality UV/vis spectra could be obtained. Compound **IEG5** was

not sufficiently soluble in the GPC eluent to obtain a significant trace, but ^{13}C NMR data was obtained in d_6DMSO .

IR data obtained from the compounds of series **II** (**IIAG1**, **IIAG2**, **IIEG1**, **IIEG2**, **IIEG3** and **IIEG5**) showed the expected strong peaks due to the carbonyl of the amide group and the nitro group of the chromophore unit. Similar shifts in the peaks due to the amide carbonyl stretching and broadening of the N-H and C-H stretching signals were observed as described previously for series **I**. ^1H NMR spectra of early generations showed the expected structure, with the benzylic CH_2 group shifted to lower ppm in the product as compared to the starting 4-nitrobenzylchloride. Problems of line broadening and solvent (DMF) entrapment became significant in higher generations. ^{13}C NMR spectra also exhibited the expected resonances. The UV/vis data shown in Table 1, was also consistent with full dendrimer end group functionalisation. Samples **IIAG1**, **IIAG2**, **IIEG1** and **IIEG2** exhibited single, sharp peaks in the GPC spectrum which comprised >85% of the sample. The remainder of the sample appeared in the form of a low molecular weight 'tail' to the main peak. **IIEG3** exhibited two poorly resolved peaks in the GPC trace; **IIEG5** showed multiple peaks (major peak 60% of the sample), indicative of growing imperfection in the dendrimer structure.

Of the compounds of series **II**, compound **IIEG7** was only superficially characterised by IR spectroscopy, due to its insolubility in organic solvents, which precluded the use of GPC or NMR or UV/vis spectroscopy.

The insolubility of the **G7** compounds of series **I** and **II** may be indicative of the approach of the starburst limiting generation¹³ for these types of functionalised dendrimers.

Due to the low solubility of the adducts of series **III**, proton NMR spectra were of a poor quality, but indicated the presence of the two tautomeric forms of the desired product. Two such functionalised dendrimers were produced, **IIIAG1** and **IIIAG2**, but due to their poor solubility (only soluble in DMF and DMSO) even at low generation, they were deemed unsuitable for further study and were not pursued.

Fluorescence Measurements. Emission and excitation fluorescence spectra of the 2-naphthyl-functionalised dendrimers, series **I** were measured and are displayed by Figures 1 and 2. The spectra of **IEG7** is also shown, but as this material was found to be extremely insoluble, only an unknown, trace amount was dissolved in solution. The model compound 2-naphthylamide (**NAM**) and 1,2-di(amido-2-naphthalene)ethane (**DINAM**) were also studied. The compounds all exhibited two main absorption peaks in their UV/visible spectra (237 nm, 280 nm) and a third weak absorption at ~320 nm. For the fluorescence studies solutions were prepared that exhibited an absorbance of 0.1 at 280 nm. It can be seen from the emission spectra that relative to **NAM**, quenching is occurring in the dendrimer systems. This indicates that non-radiative pathways not available to the model chromophore in methanol solution are open to the dendrimer molecules. This is assumed to be attributable to intramolecular associations. Chromophores on each of the dendrimer molecules may be held closer and are less solubilised than the free chromophores. Further, a broadening of the low energy side of the emission peak is observed in the lower generation dendrimers. This may be due to the formation of excimer species, made possible in the lower generations by the flexible branches which could allow the necessary conformation to occur. Similar quenching effects and the observation of excimer species have recently been reported in naphthyl-terminated polyether dendrons¹⁴.

EFISH Measurements. The study of mesoscopic molecules using the technique of EFISH¹⁵ represents a new area of interest. The recent study of 4-dimethylaminophenylcarboxamide functionalised poly(propyleneimine) dendrimers by Hyper-Rayleigh Scattering¹⁶ showed the expected flexible, averaged sphere-like structure at lower generations, which tends towards a rigid spherical morphology at higher generations.

The models conventionally used to analyse the EFISH results assume that individual dipolar solute molecules behave independently of each other and the values of $\mu\beta$, the measure of microscopic second order nonlinearity obtained, rely on the assumption that the largest perturbation to the symmetry of the system arises through the interaction of the applied field with a Boltzmann population of such species. In the case of dendrimer particles as described here, there are a number of terminal dipolar groups attached to each other through long flexible linkages. The groups may have independent freedom to reorient (as they appear to do in macroscopic side-chain polymer systems) but one cannot expect the system to exhibit translational uniformity. At the concentrations used in these preliminary experiments there is scope for dipolar associations either between moieties on the same molecule or between dendrimer molecules. In addition, the structures may have restrictions to free rotations because of the hydrogen bonding amido groups in the branches. The analysis therefore is made at the simplest level possible. The second harmonic coefficients (d) are measured at a single concentration and a subtraction of the d coefficient of the solvent is made. At this level, there is no need to invoke a number density of either molecules or dipolar terminal groups since no attempt is made to assign a $\mu\beta$ product.

The two molecules studied were **IIAG1** and **IEG1**. Dendrimer **IIAG1** contains 6 terminal nitro benzyl groups whereas, **IEG1** contains 8 terminal groups.

Interestingly, in comparison of Maker fringes obtained from **IIAG1** in dichloromethane and the pure solvent, the spacing between the fringes for the solution are longer than for the pure solvent they are also somewhat less intense (absorption corrections are made in the analysis). This indicates an increase in coherence length (approximately 22 μm compared with 17 μm obtained for dichloromethane). A similar, and larger, effect was seen for **IEG1** where the coherence length extended to 28 μm despite the molar concentration being lower than for **IIAG1** (although the weight fraction is the same).

A complete study would involve taking data at a number of different concentrations and extrapolating to infinite dilution. The equations used in such an analysis include the term $-\frac{\delta n^2}{\delta w}$, which accounts for the refractive index change with weight fraction, w , of solute. Other terms take account of the volume fraction of the two components. Our evidence of the coherence length change does show that these terms might become important in the case of dendrimer solutions whereas it can be satisfactorily neglected in the case of small molecule studies. Clearly the simple rule of additivity of coefficients neglecting the volume fractional changes with composition is insufficient to explain the results. The lower intensity of the Maker fringes obtained from solutions might be associated with the dielectric loss observed at 1 Mhz¹⁷ (the EFISH voltage pulse widths are 6 μs).

Conclusions

We have produced a range of chromophore functionalised dendrimers, although at higher generations dislocations in the structure are evident from GPC and NMR studies. The compounds described herein have been investigated by fluorescence spectroscopy which showed the expected feature of increased energy transfer with

higher generations. However, the EFISH results of these initial studies reveal unexpected effects due to the attachment of the chromophore to the dendrimer core.

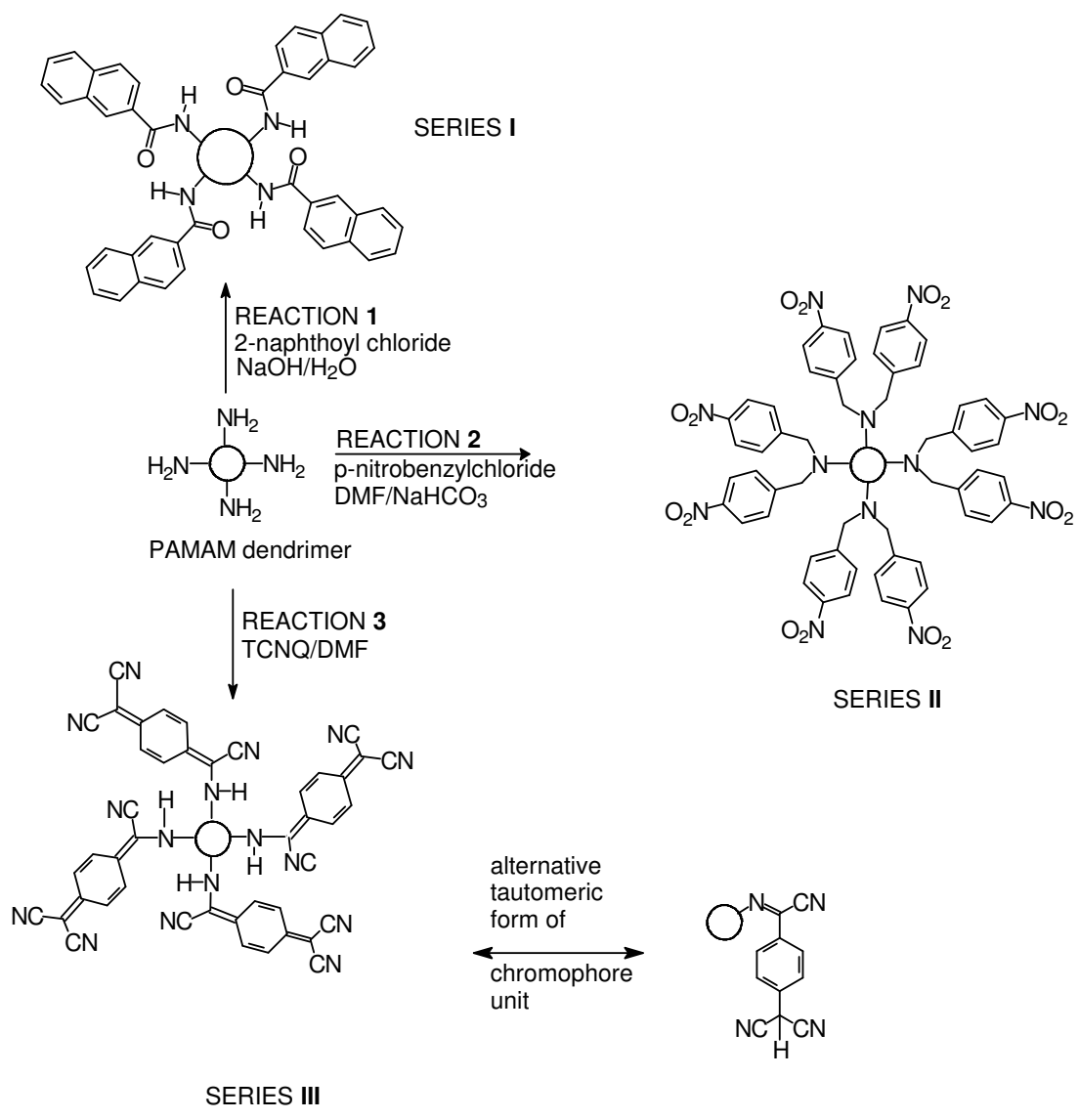
A more detailed study would be required to fully understand such phenomena.

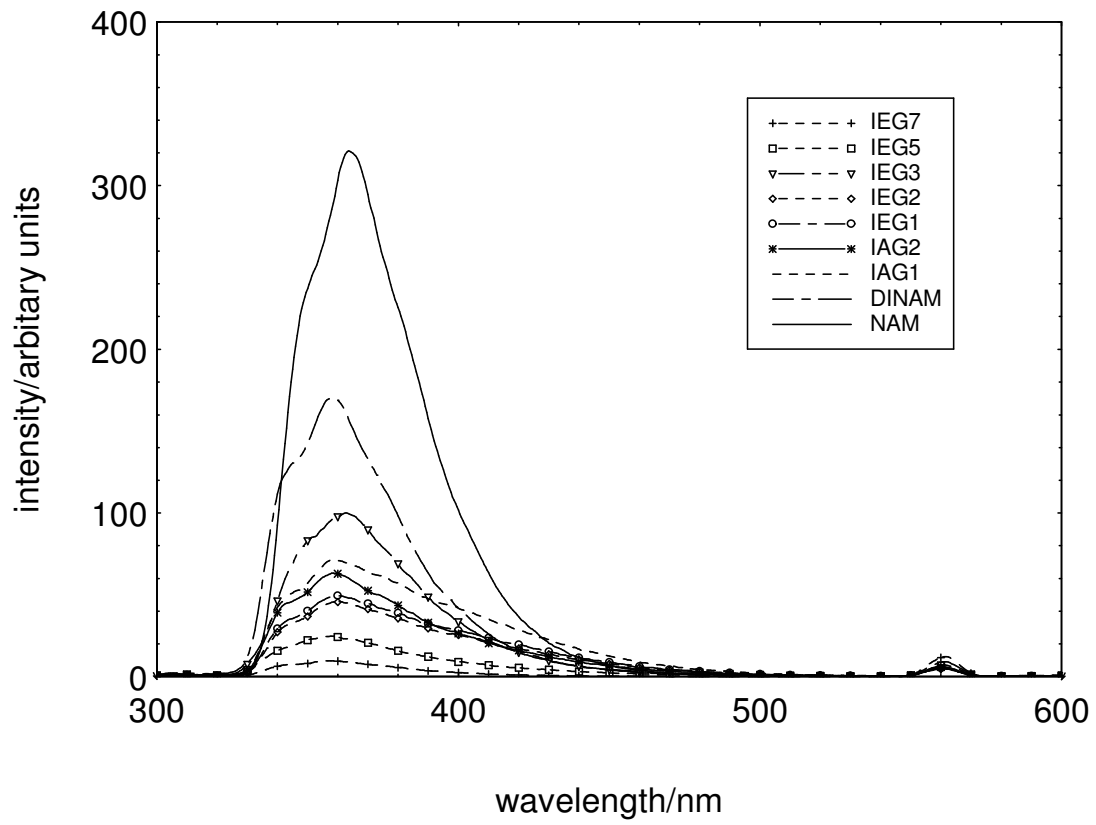
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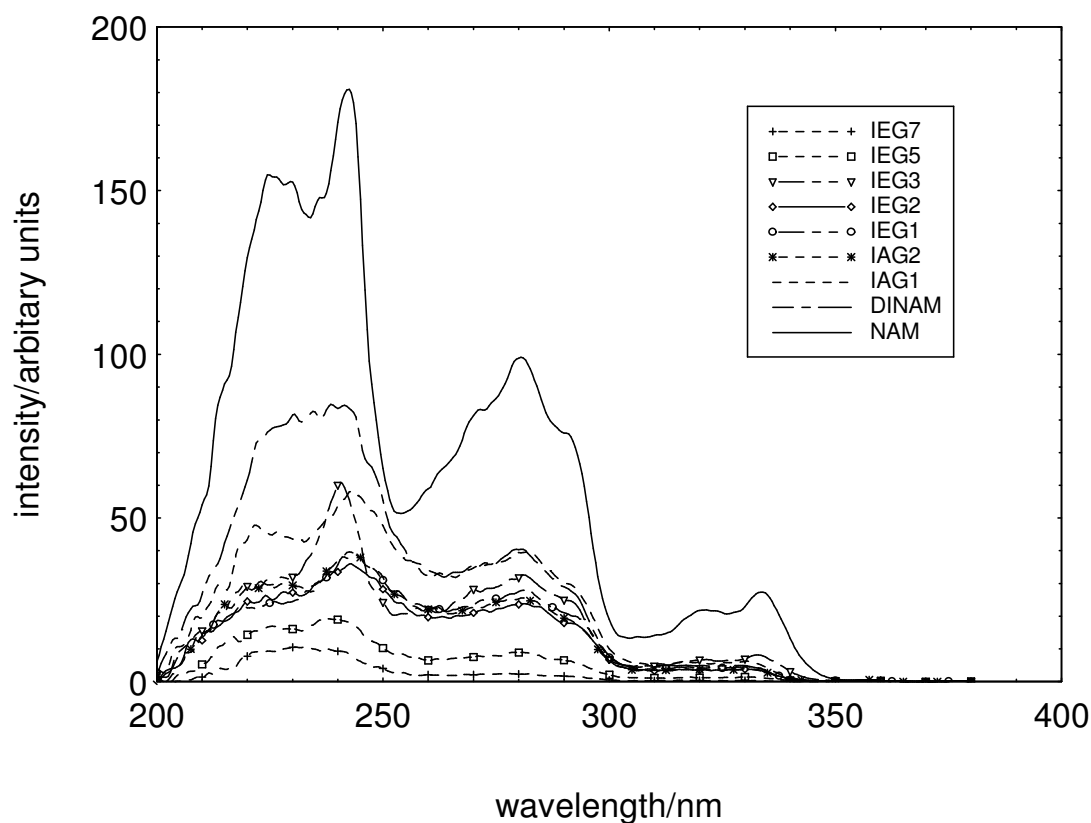
compound	Extinction Coefficient	no. of chromophore units per dendrimer molecule
IAG1	11000	3
IAG2	17000	6
IEG1	10000	4
IEG2	15000	8
IEG3	35000	16
IEG5	140000	64
IIAG1	58000	3
IIAG2	98000	6
IIEG1	130000	8
IIEG2	270000	16
IIEG3	500000	32
IIEG5	4700000	64

sample	RMM expected	RMM found	polydispersity
IEG1	1133	1900	1.2
IEG2	2663	4600	1.2
IEG3	5722	6890	1.2
IEG4	11837	11000	1.5
IIAG1	1169	1200	1.3
IIAG2	2663	2600	1.2
IEG1	1597	1500	1.5
IEG2	3590	5000	1.9
IEG3	7576	11000	-
IEG5	31495	30000	-

- Scheme 1 Outline synthesis schemes for the dendrimers studied.
- Figure 1 Fluorescence emission spectra ($\lambda_{\text{ex}} = 280 \text{ nm}$) of Series **I**.
- Figure 2 Fluorescence excitation spectra at 400 nm of Series **I**.
- Table 1 Uv/vis data obtained for the functionalised dendrimers studied.
- Table 2 GPC data obtained for the functionalised dendrimers studied.







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