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Hydrophobic dendrimer-derived nanoparticles

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Abstract

Lipidic polylysine dendrimers, synthesized using Fmoc solid phase peptide techniques, have been formulated as nanoparticles by precipitation from solution in dichloromethane. The effect of concentration on the diameter and stability of nanoparticles formed from two short homologous series of dendrimers – one fifth generation and one sixth generation series and with surface C_4, C_{10} or C_{12} groups – was investigated using photon correlation spectroscopy. The increase in generation from fifth to sixth resulted in increased diameter for each chain length. An increase in the surface lipidic chain length from C_4 to C_{12} had no effect on the particle diameter of aggregates derived from fifth generation dendrimers, and a small and variable effect on the sixth generation derived nanoparticles. Using pyrene (excitation 340 nm) as a hydrophobic fluorescent probe, a decrease in intensity peak I_1 (374 nm)/I_3 (385 nm) in the emission spectra (340–600 nm) was observed in the two dendrimers studied, fifth generation dendrimers with C_{10} or C_{12} surface lipidic chains, as the dendrimer concentration increased, reaching a plateau at higher concentrations, indicating that a more compact form of the aggregates with a more hydrophobic interior was obtained. Apart from the hydrophobicity of the dendrimers and dendrimer concentration, the flexibility of the dendrimers might have a significant effect in determining nanoparticle size. The aggregates derived from the fifth generation dendrimers with C_{10} or C_{12} surface lipidic chains are stable in purified intestinal fluid but not in purified stomach fluid, in which further aggregation of the nanoparticulate dendrimer aggregates occurs as an effect of pH, salts, proteins and enzymes in these fluids. This study demonstrates, inter alia, the importance of testing nanoparticulate delivery systems in relevant physiologically based fluids prior to their use in vivo.

Keywords: Dendrimers; Nanoparticles; Aggregation

It has been well documented that physical characteristics including size and surface nature affect nano- and micro-particulate translocation in vivo. Generally, the smaller the diameter the greater the extent of absorption, charged surfaces reduce particle absorption while increasing hydrophobicity results in greater uptake and translocation (Florence, 1997). However, several other factors are also of importance when using nanoparticles derived from lipidic subunits of individual dendrimers, such as described in the present paper. Li et al. (2004) found the stability of dendrimer aggregates to be dependent on the structural flexibility

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of the individual dendrimer molecules. The nature of the packing into the aggregates affects the size, shape, size distribution and colloidal stability of the formed particles and are factors which determine drug entrapment and the flow properties of the dispersed particles (Luckham and Ukeje, 1999).

Dendrimers are three dimensional hyper-branched macromolecules (Tomalia et al., 1990) with great potential as carriers because their surface properties can be modified to meet a variety of design criteria. Even though many structurally different dendrimers have been synthesized, the full potential of dendrimers as drug delivery vectors has yet to be fulfilled. If dendrimers are to be used as delivery systems, it is important to understand the nature of packing during self-assembly and controlled aggregation with and without encapsulated drug molecules. Studies have been conducted on interactions of cationic dendrimers with DNA (Ramaswamy et al., 2003), complexation or interaction of piroxicam (Wiwattanapatapee et al., 1999) and ibuprofen (Milhem et al., 2000) and the formation of higher order structures (Al-Jamal et al., 2003). Many of the dendrimers studied have been cationic dendrons, which, because of their positive surface charges, are likely to interact with charged molecules and membranes (Singh et al., 2003). More hydrophobic dendrimeric systems might avoid inadvertent interactions with anionic molecules, but may possess other characteristics. The hydrophobic dendrimers discussed here aggregate or can be induced to form nanoparticles, which must consist of large numbers of individual dendrimers. In this communication, we report on the nature of the aggregates and their physical stability in biological fluids.

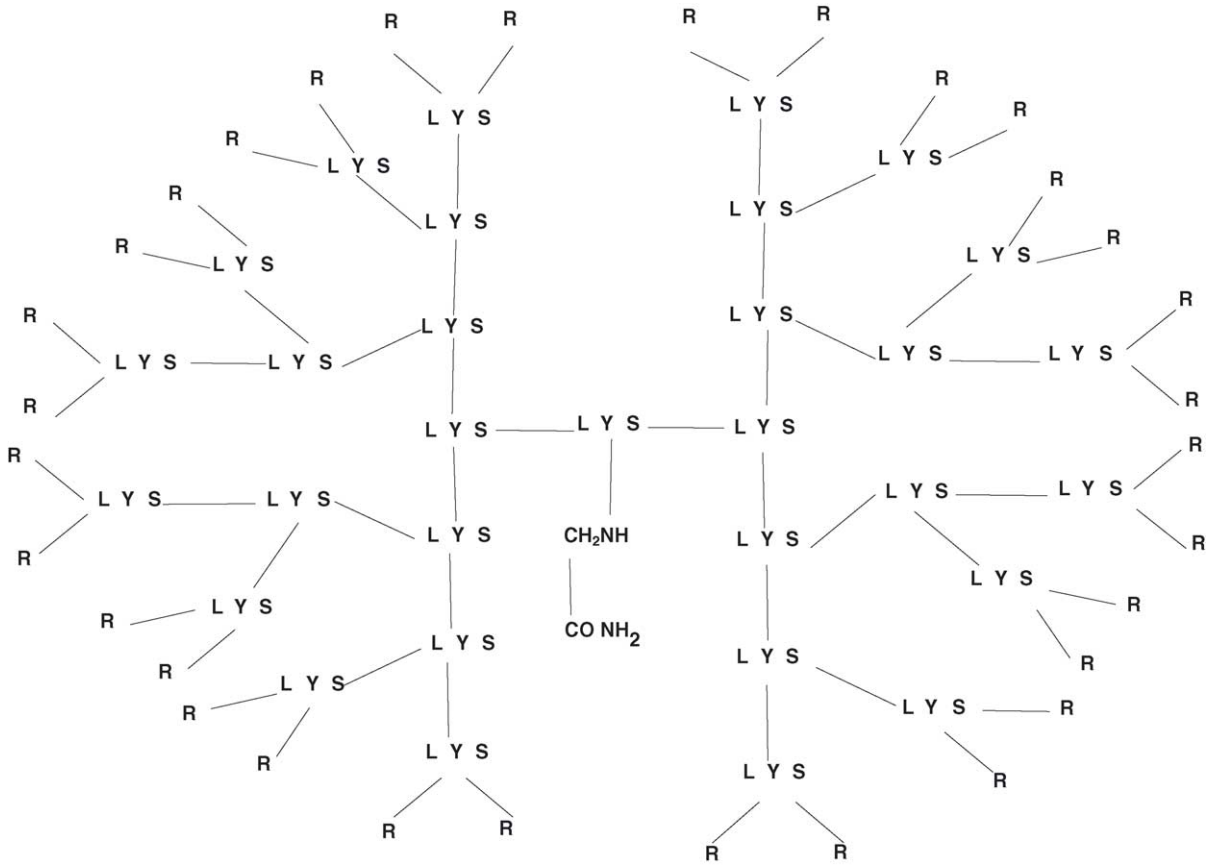

Fig. 1. Structural diagram of the dendrimers studied: R = butyric acid (C4), decanoic acid (C_{10}), dodecanoic acid (C_{12}), dendrimers **I**, **II**, **III** respectively or lys-(butyric acid $(C_4))_2$, lys-(decanoic acid $(C_{10}))_2$, lys-(dodecanoic acid $(C_{12}))_2$, respectively in dendrimers **IV**, **V** and **VI**.

A series of lipidic polylysine dendrimers (Fig. 1) were prepared by using Fmoc solid phase peptide synthesis. The dendrimers, with molecular weights ranging from ∼6200 to 19,800, differed in generation and length of lipidic chain (Table 1). Controlled aggregates of the dendrimers (**I–VI**) were formed using a precipitation method employed by Quintanar-Guerrero et al. (1998). Each dendrimer (**I–VI**) (0.25 mg) was solubilised in dichloromethane (DCM) (0.25 ml) and double distilled deionised water (3.5 ml) added. The solution was sonicated and the emulsion formed was stirred until the DCM evaporated and the dendrimer aggregates precipitated. The effect of dendrimer concentration on apparent particle size and stability was investigated using photon correlation spectroscopy (PCS) (Malvern Instruments 5000, UK) and transmission electron microscopy (TEM). Packing of the dendrimers was investigated using pyrene as a hydrophobic fluorescent probe (excitation, 340 nm) (Perkin-Elmer, Spectrophotometer LS 50B, UK). The intensity (peak height) ratios of the first band (374 nm) to the third band (385 nm) (I_1/I_3) in the emission spectra (350–600 nm) were determined as a function of dendrimer concentration. The stability of the dendrimer aggregates was assessed in purified (rat) intestinal and stomach fluid. The small intestine was separated from the stomach and the large intestine, flushed through with double distilled deionised water (15 ml) and the content centrifuged and filtered sequentially through 0.6 and 0.2 μm filters. The stomach was cut open and the content washed in pure water (15 ml), centrifuged and filtered through 0.6 and 0.2 μm filters.

Using the same amount of dendrimer (0.071 mg/ml) and the same volume (0.25 ml DCM) of the organic phase, the effect on the Z-average and number average diameter (nm) of batches of particles prepared from dendrimers **I–VI** was assessed (Fig. 2). Compounds **I**, **II** and **III** are fifth generation dendrimers with surface chains C_4, C_{10} and C_{12}, respectively, while **IV**, **V** and **VI** represent the sixth generation dendrimers with C_4, C_{10} and C_{12} surface chains, respectively. A slight increase in the Z-average diameter was observed when comparing fifth generation with sixth generation dendrimers: **I** with **IV**, **II** with **V** and **III** with **VI**. A similar trend was found when comparing the mean number diameters of fifth and sixth generation dendrimers: **I** (26 nm) with **IV** (57 nm), **II** (27 nm) with **V** (85 nm) and **III** (25 nm) with **VI** (48 nm). More than 98.5% of all particles from the fifth generation dendrimers were

Table 1
Lipidic polylysine dendrimers **I–VI** nomenclature, molecular weight and diameter (molecular modelling)

Number	Nomenclature	Molecular weight (kDa)	Dendrimer diameter (nm)
I	$GlyLys_{31}(C_4)_{32}$	6275	3.8
II	$GlyLys_{31}(C_{10})_{32}$	8968	4.5
III	$GlyLys_{31}(C_{12})_{32}$	9865	4.8
IV	$GlyLys_{63}(C_4)_{64}$	12620	5.0
V	$GlyLys_{63}(C_{10})_{64}$	18006	5.8
VI	$GlyLys_{63}(C_{12})_{64}$	19802	6.0

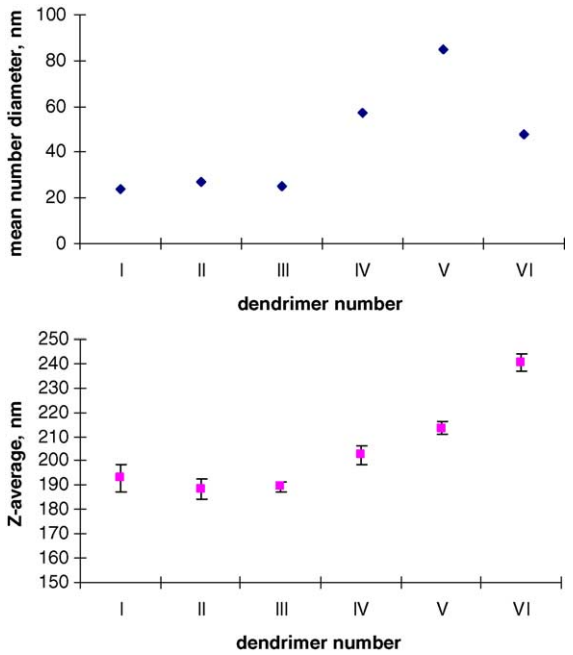

Fig. 2. Effect on apparent particle diameter (nm) of batches prepared from dendrimers **I** → **VI**, **I**, **II** and **III** are fifth generation dendrimers with surface chains C_4, C_{10} and C_{12} while **IV**, **V** and **VI** represent the sixth generation dendrimers with C_4, C_{10} and C_{12}. The concentration of dendrimers was 0.72 mg/ml. Comparing fifth and sixth generation of dendrimers: **I** (192 ± 6 nm) with **IV** (204 ± 3.8 nm); **II** (189 ± 4 nm) with **V** (213 ± 3 nm); and **III** (190 ± 2 nm) with **VI** (240 ± 3.7 nm), an increase in Z-average and mean number values of the dendrimer-derived nanoparticles was observed. An increase in the surface lipid lipidic chain length from C_4 to C_{12} had no effect on the particle diameter of aggregates derived from fifth generation dendrimers, and a small and variable effect on the sixth generation derived nanoparticles. Using similar molar ratios extrapolated from Fig. 3 did not alter the graph significantly.

less than 100 nm in diameter, whereas the sixth generation dendrimers showed dendrimer **IV** and **VI** to have around 90%, and dendrimer **V** around 70% of all particles less than 100 nm. This suggests that changing the dendrimer from fifth to sixth generation increased the number of larger aggregates. The increase in the number of lipidic chains on the dendrimers, from 32 to 64, seems to cause this increased aggregation.

Increase in the carbon chain length as in dendrimers **I**–**III** had no effect on the Z-average diameter of the nanoparticles (<190 nm). Dendrimers **IV**–**VI** form particles with measured diameters from 204 ± 3.8 nm to a maximum of 240 ± 3.7 nm when the lipidic chain was increased (C_4–C_{12}). The increase in Z-average diameter of dendrimers **IV**–**VI** was caused by the aggregation, and hence polydispersity of the nanoparticles. The sixth generation dendrimer with C_{10} lipidic chains attached (dendrimer **V**) had fewer particles (∼70%) under 100 nm and a larger mean number diameter (85 nm) than the other members of the same generation. This trend was not observed for the fifth generation dendrimers. Only as the number of lipidic chains on the dendrimer increased, does the difference become more significant. How exactly the C_{10} lipidic chain, compared to the C_4 and C_{12}, changed the formation of the aggregates is not clear. Transmission electron microscopy indicates that the apparent particle diameter of the aggregates is in the range ∼20–50 nm, similar to the mean number averages.

To further study the packing of the dendrimers, the effect of dendrimer concentration on particle diameter and the hydrophobicity of their aggregates (using pyrene as hydrophobic fluorescent probe) were studied. Particles of dendrimer **I** showed an increase in mean number diameter from ∼30 to ∼50 nm (which represents a 462% increase in volume) as the dendrimer concentration increased from 0.07 to 1 mg/ml, whereas dendrimers **II** and **III** showed a slight decrease in Z-average diameter and mean number diameter over the concentration range 0.07–0.45 mg/ml (Fig. 3). The increase in amount of small particles in the systems (<100 nm) led to this decrease in particle diameter. These results suggest that dendrimers with surface lipidic chains (C_4–C_{12}) are likely to form condensed aggregates as the dendrimer concentration increased, leading to either a larger (dendrimer **I**) or a smaller (dendrimers **II** and **III**) particle diameter. The packing seemed mainly governed by hydrophobic interactions

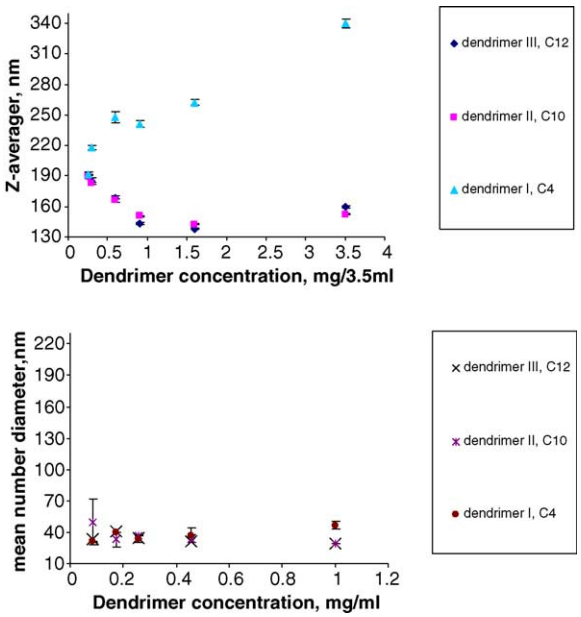

Fig. 3. Effect of dendrimer (**I**, **II** and **III**) concentration on particle diameter (Z-average and mean number diameter) (nm) of the aggregates using PCS. Increasing the concentration of dendrimers **II** and **III** resulted in a decrease in Z-average (∼190 to ∼160 nm) and mean number diameter (∼40 to ∼20 nm). Dendrimer **I**, however, showed an increase in Z-average (192–339 nm) and mean number diameter (∼30 to ∼50 nm) as the concentration of dendrimer increased.

between the lipidic chains on the dendrimer surfaces. However, the flexibility of the surface structures of the dendrimers on packing might be the reason that the dendrimer with the C_4 lipidic chain behaves differ-

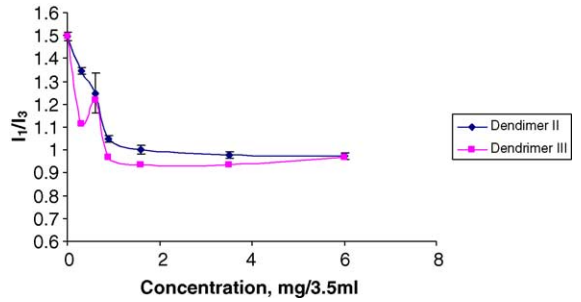

Fig. 4. The effect of dendrimer (**II** and **III**) concentration on the I_1/I_3 ratios was investigated using pyrene (excitation, 340 nm) as a hydrophobic fluorescent probe. The intensity (peak height) ratios of the first band (374 nm) to the third band (385 nm) (I_1/I_3) in the emission spectra (350–600 nm) were determined as a function of dendrimer concentration.

ently from the dendrimers with longer lipidic chains (dendrimers **II** and **III**).

Pyrene (excitation, 340 nm) was the hydrophobic fluorescent probe; the intensity (peak height) ratios of the first band (374 nm) to the third band (385 nm) (I_1/I_3) in the emission spectra (350–600 nm) were determined as a function of dendrimer (**II** and **III**) concentration (Fig. 4). Results showed a decrease in I_1/I_3, indicating an increase in non-polar nature of the dendrimer aggregates. A plateau is reached at the higher concentration suggesting that a compact form of the aggregates was attained. The stability of the dendrimer-derived aggregates was not only dependent on surface properties of the dendrimer but also on dendrimer concentration.

Amphiphilic dendrons have been shown to form aggregates when they come in contact with cell culture media (Singh et al., 2003), posing problems for interpretation of cellular interactions. Nanoparticles from dendrimers (**II** and **III)** (6 mg) were prepared as before in 0.25 ml dichloromethane and 3.5 ml water. These (150 μl from a 1.71 mg/ml solution) were then incubated with intestinal fluid (2 ml) and stomach fluid (2 ml) and the apparent particle size measured by PCS over 180 min. The pH of intestinal and stomach fluid was 6.5 and 4.8, respectively. The results (Fig. 5a) showed that over a period of 3 h, the dendrimers were stable in intestinal fluid, whereas on incubation in stomach fluid, there was an immediate increase in apparent particle diameter (∼2000–3000 nm) (Fig. 5b).

We have formulated a nanoparticulate system using a range of dendrimers with increasing hydrophobicity. Hydrophobic interaction of the lipidic chains on the dendrimer is the likely driver of aggregate formation and stability. Increasing the dendrimer generation, from the fifth to the sixth, caused the formation of larger nanoparticles. Increasing the dendrimer concentration resulted in either an increase or decrease in particle diameter depending on the length of lipidic chains, suggesting that structural flexibility also effects the aggregation of these particles. Aggregation of the systems in intestinal fluid is likely to affect the absorption of these systems—a phenomenon under study at present.

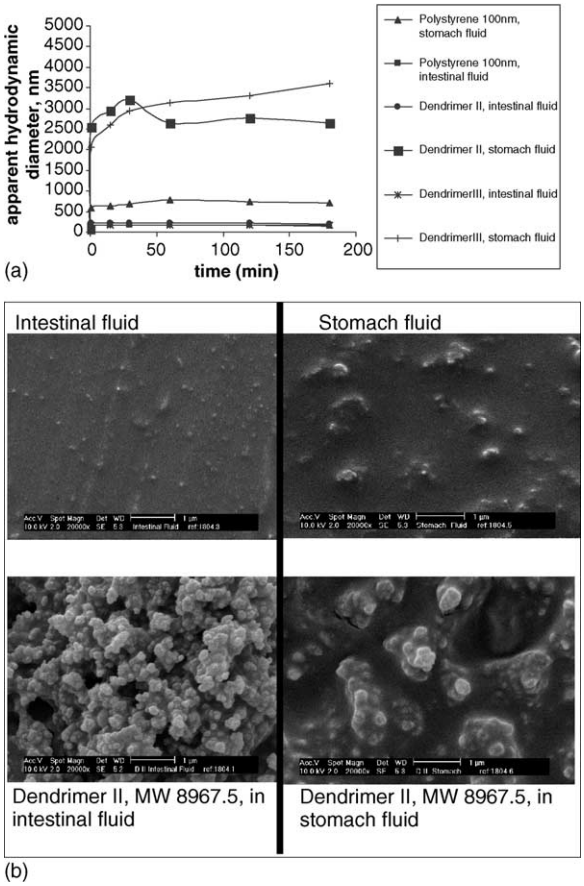

Fig. 5. (a) A plot showing the effect over a 3 h time period of purified intestinal and stomach fluid on the apparent diameter of aggregates prepared from dendrimers **II** and **III**. No significant increase in apparent particle size was observed in intestinal fluid (∼220 nm). In purified stomach fluid, an immediate and significant increase in apparent particle size was found (∼2000–3000 nm). (b) SEM pictures of purified intestinal and stomach fluid with and without dendrimer **II** (1.71 mg/ml) after 3-h incubation.

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