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The effect of PAMAM dendrimer generation size and surface functional group on the aqueous solubility of nifedipine

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Abstract

The aim of this study was to investigate the effect of low generation (G0–G3) ethylenediamine (EDA) core poly(amidoamine) (PAMAM) dendrimers on the aqueous solubility of nifedipine. The aqueous solubility of nifedipine was measured in the presence of dendrimers at 30 °C in Tris buffers at pH 4, 7, and 10 using the traditional rotary bottle method. Results showed that generation size, surface functional group and the pH of the aqueous media determined the aqueous solubility of nifedipine and that solubility profiles were of the Higuchi A_L-type. Both amine and ester terminated dendrimers caused the highest increase in nifedipine solubility at pH 7. The order in which the dendrimers increased the solubility at pH 7 was G2.5 > G3 > G1.5 > G2 ≥ G0.5 > G1 > G0. In addition, at each pH, the solubility of nifedipine was greater in the presence of ester-terminated dendrimers compared to the amine-terminated dendrimers possessing the same number of surface functional groups. The pH and surface functional group dependent increase in nifedipine solubility caused by the dendrimers was likely due to changes in the degree of protonation of the dendrimers. A decrease in the protonation of dendritric amines was expected to promote hydrogen bond formation between the tertiary amines within the dendrimer cavity and the nifedipine molecule.

Keywords: Solubilization; Nifedipine; PAMAM dendrimers; Complexation

1. Introduction

Nifedipine (4-(2-nitrophenyl)-2,6-dimethyl-3,5-dicarbomethoxy-1,4-dihydropyridine) (Fig. 1) is a calcium channel blocking agent. A major problem associated with the formulation and effectiveness of the nifedipine is its poor aqueous solubility, 5–6 μg/ml over a pH range of 4–13, which may account for its low and irregular bioavailability in humans (Boje et al., 1988; Ali, 1989; Vippagunta et al., 2002). Several techniques such as solubilization by surfactants, co-solvents, complexing agents, and crystal modification have been used to increase the solubility of nifedipine (Emara et al., 2002). Of these techniques,

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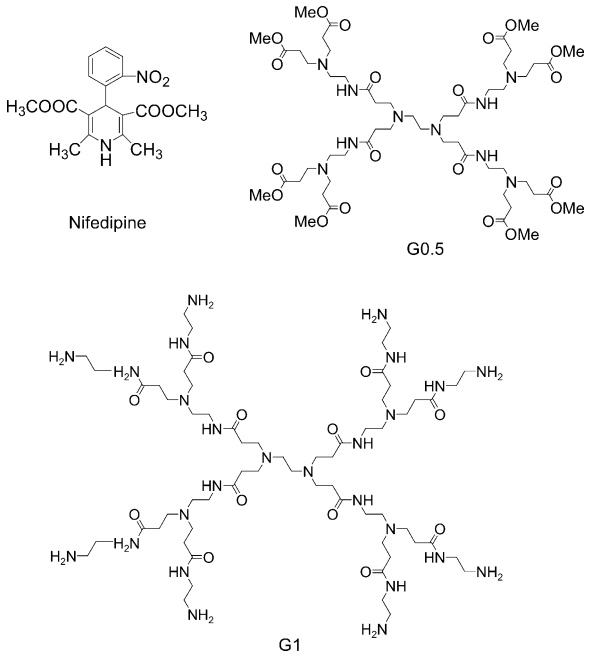


Fig. 1. Molecular structures of nifedipine and PAMAM dendrimers with ester (G0.5) and amine (G1) terminated surface functional groups.

solubilization and complexation of nifedipine have been investigated using a variety of complexing agents such as salicylates (Kleinbloesem et al., 1984), phenolic ligands (Boje et al., 1988), and β- and hydroxypropyl β-cyclodextrin (Chowdary and Reddy, 2002). The objective of this study was to examine the enhancement of the aqueous solubility of nifedipine using ethylenediamine (EDA) core poly(amidoamine) (PAMAM) dendrimers as solubility enhancers.

Dendrimers are synthetic, highly branched, spherical, mono-disperse macromolecules (Tomalia et al., 1985; Frechet, 1994). A typical dendrimer consists of three basic components: (a) a *central core* from which the polymeric branches emanate; (b) *repeat units* the nature of which determines the microenvironment of the interior and in turn the solubilization ability of the dendrimer; and (c) the *terminal groups*, the nature and number of these groups are mainly responsible for the behavior of dendrimers in solution (Tomalia et al., 1985).

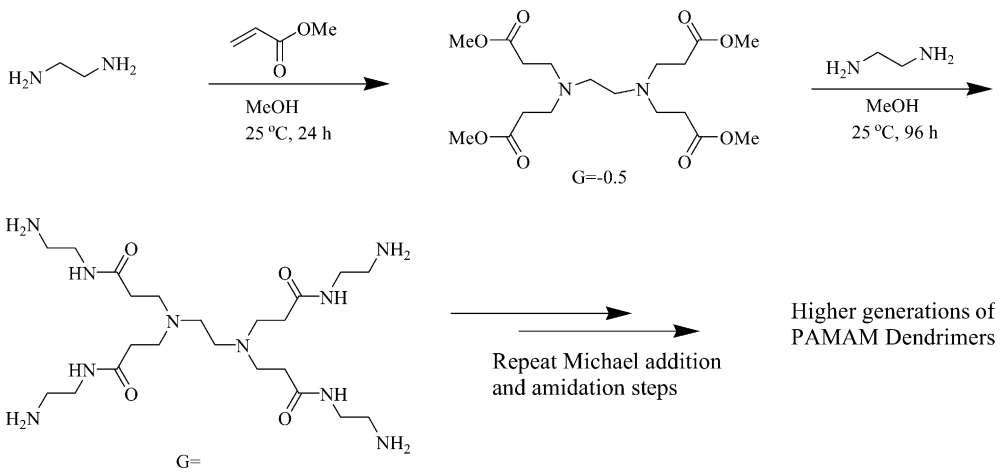
The family of dendrimers most investigated in drug delivery is the PAMAM dendrimers (Patri et al., 2002). PAMAM dendrimers are biocompatible, non-immunogenic, water-soluble, and possess terminal-modifiable amine functional groups for binding various targeting or guest molecules. The internal cavities of dendrimers can host metals or guest molecules because of the unique functional architecture, which contains tertiary amines and amide linkages (Esfand and Tomalia, 2001; Patri et al., 2002). Generation 3 (G3) and generation 4 (G4) PAMAM dendrimers have been used to encapsulate and solubilize acidic drugs such as piroxicam (Wiwattanapatapee et al., 1999), ibuprofen (Kolhe et al., 2003) and indomethacin (Chauhan et al., 2003). Recently, Kojima et al. (2000) modified G3 and G4 PAMAM dendrimers by attaching poly(ethylene) glycol grafts and successfully encapsulated methotrexate (MTX; ∼25 molecules) and adriamycin (ADR; ∼6 molecules). Twyman et al. (1999) and Beezer et al. (2003) converted the ester terminated half-generation (G1.5 and G2.5) PAMAM dendrimers to a more soluble hydroxyl surface by reacting with Tris and used to solubilize small acidic hydrophobic guest molecules. Other uses of the dendrimers are the delivery of DNA and oligonucleotides (Poxon et al., 1996), the development of targeted delivery systems, and as carriers for the delivery of drugs via the gastrointestinal tract with cationic PAMAM dendrimers of G3 and G4, and anionic PAMAM dendrimers of G2.5, G3.5, and G5 (Wiwattanapatapee et al., 2000).

Although the lower generation dendrimers (G < 4) are less sensitive to conformational changes with variations in the pH, non cytotoxic and stable in the acidic medium, they have not been extensively studied to determine their solubilization potential (Chen et al., 2000; El-Sayed et al., 2002). In the present work the effect of lower generation ethylenediamine core PAMAM dendrimers on the aqueous solubility of the hydrophobic drug nifedipine was studied. The effect of both amine terminated full generation (G0–G3) and ester terminated half-generation (G0.5–G2.5) PAMAM dendrimers on the aqueous solubility of nifedipine was studied.

2. Materials and methods

2.1. Materials

Nifedipine was purchased from Sigma Chemical Company (St. Louis, MO, USA). Ethylenediamine, methylacrylate, methanol (HPLC grade), sodium dihy-



Scheme 1. Divergent growth strategy for the synthesis of (PAMAM) dendrimers (Esfand and Tomalia, 2001).

drogen phosphate, citric acid, tromethamine, sodium borate, potassium chloride, and standard pH buffers were obtained from Spectrum Chemical Company (Gardena, CA, USA). For both solubility studies and HPLC analysis, distilled deionized water was used.

2.2. Synthesis of PAMAM dendrimers

Ethylenediamine core PAMAM dendrimers were synthesized using Tomalia's divergent growth approach (Scheme 1, Esfand and Tomalia, 2001). The synthesis involves two consecutive chain-forming reactions, the exhaustive Michael additions reaction, and the exhaustive amidation reaction, repeating alternatively. Michael addition of methyl acrylate to ethylenediamine in methanol gives the ester terminated half-generation dendrimers designated, G0.5. The exhaustive amidation reaction of ester-terminated dendrimers with large excess of ethylenediamine in methanol produce amine terminated full generation dendrimers referred to as Gn. Repetition of Michael addition and amidation reactions gives next higher generation dendrimers. In the present study, both amine-terminated full generation (G0–G3) and ester-terminated half-generation (G0.5–G2.5) PAMAM dendrimers were synthesized and used in the solubilization studies of nifedipine.

2.3. HPLC analysis of nifedipine

Nifedipine was analyzed by high performance liquid chromatography (AS 1000 autosampler and P2000 pump, Thermo Separation Products, Waltham, MA) equipped with a multiple wavelength UV detector (UV 3000 detector) set at a wavelength of detection λ_{max} = 254 nm. Chromatographic separation was performed using a C_{18} column (Econosil, 5 μm particles, 250 mm × 4.6 mm, Alltech, Deerfield II). The mobile phase was methanol:water (2:1, v/v); flow rate 0.7 ml/min; injection volume 20 μl. The retention time for nifedipine was 11 min, the limit of detection was 1.0 ng/ml. The reported results represents the means of three analyses, and the solutions were protected from light to prevent photo degradation of nifedipine. The HPLC method used in this study complied with specifications for precision, accuracy, selectivity, linearity, and ruggedness as required by the USP XXIV (2000).

2.4. Solubility measurements

The aqueous solubility of nifedipine in Tris buffers (composition: different ratios of citric acid 21.01 g/l, potassium dihydrogen phosphate 16.61 g/l, sodium tetraborate 19.07 g/l, and tromethamine 12.11 g/l solutions) at pH 4, 7, and 12 was determined in the presence of increasing concentrations of the dendrimers. The ionic strength of the buffer was maintained constant at 1.0 M using potassium chloride. Solubility studies were carried out using the Higuchi rotating bottle method (Higuchi and Conners, 1965). An excess of nifedipine was added to 3 ml buffer solutions in 5 ml screw-capped amber colored vials containing in-

Table 1
Theoretical characteristics of ethylenediamine core PAMAM dendrimers (Esfand and Tomalia, 2001)

Generation	Molecular weight (Da)	Diameter (nm)	No. of tertiary amines	No. of surface groups	Surface group
0	517	1.4	2	4	$–NH_2$
1	1430	1.9	6	8	$–NH_2$
2	3256	2.6	14	16	$–NH_2$
3	6909	3.6	30	32	$–NH_2$
0.5	1207	–	6	8	$–COOCH_3$
1.5	2809	–	14	16	$–COOCH_3$
2.5	5978	–	30	32	$–COOCH_3$

(–) not available.

creasing amounts of the dendrimers. The vials were rotated at 60 rpm while being kept at 30 ± 1.0 °C. Preliminary experiments indicated that a duration of 6 h provided sufficient time to reach equilibrium. After 6 h, samples were filtered through a 0.45 μm cellulose acetate filters (Osmonics Inc., Minnetonka, MN), diluted appropriately with the mobile phase and analyzed by HPLC. Measurement of pH at the end of the solubility studies showed no significant alterations in the pH of the medium. Phase solubility diagrams were constructed by plotting the molar concentrations of nifedipine (solubility) versus molar concentration of dendrimers. From these plots, the stability constants for the complexation of nifedipine with dendrimers were calculated (Higuchi and Conners, 1965; Yalkowsky, 1999).

2.5. Statistical analysis

In this study, a three-factor factorial design (De Muth, 1999) with $n = 3$ replicates was used where the aqueous solubility of nifedipine was measured for three levels of factor A (pH of the medium: 4, 7, and 10); two levels of factor B (surface functional group of dendrimers: NH_2 and $COOCH_3$); and three levels of factor C (generation size of the dendrimers: 1–3). Using this design, the effect of three factors on the aqueous solubility of nifedipine was evaluated at a 0.05 significance level using a commercial software package (Student Statistix 7.0, Analytical Software, Tallahassee, FL). In the presence of significant interaction among the factors, a one-way ANOVA was used. Finally the differences between the two sample means were observed by pair wise comparisons using a least significant difference (LSD) test performed with SPSS 10.0 for Windows (SPSS, Chicago, IL).

3. Results and discussion

The solubility of nifedipine in the Tris-buffers were 1.28 ± 0.20 M ($\times 10^{-5}$) (mean ± S.D., $N = 21$) over the pH range of 4–10. These values agreed well with the previous reports on the solubility of nifedipine (Boje et al., 1988; Ali, 1989; Vippagunta et al., 2002; Chowdary and Reddy, 2002). Since nifedipine is a weak acid with pK_a of 13 its solubility did not increase significantly with an increase in the pH of the aqueous medium from pH 4 to 10 (Ali, 1989).

3.1. Structural characterization of the PAMAM dendrimers

The PAMAM dendrimers were characterized structurally via ^1H and ^{13}C NMR, IR, and mass spectral analysis, Table 1, and the results agreed with that reported in the literature (Esfand and Tomalia, 2001). Since the dendrimers are highly hygroscopic, they were stored as 10% (w/w) solutions in methanol. As shown in Table 1 molecular weight and number of peripheral groups of dendrimers increase exponentially with each generation, while the diameter increased more or less linearly (Esfand and Tomalia, 2001). This means with each ensuing generation the surface density of peripheral moieties, primary amines (in full generation dendrimers) and ester groups (in half-generation dendrimers), increases. For example, G0 generation dendrimers have 4 primary amine surface functional groups where as G1–G3 have 8, 16, and 32 surface groups, respectively. Similarly, G0.5, G1.5, and G2.5 have 8, 16, and 32 ester terminated surface functional groups, respectively. Examples of the molecular structures of the ester (G0.5) and the amine (G1) terminated PAMAM dendrimers are shown in Fig. 1.

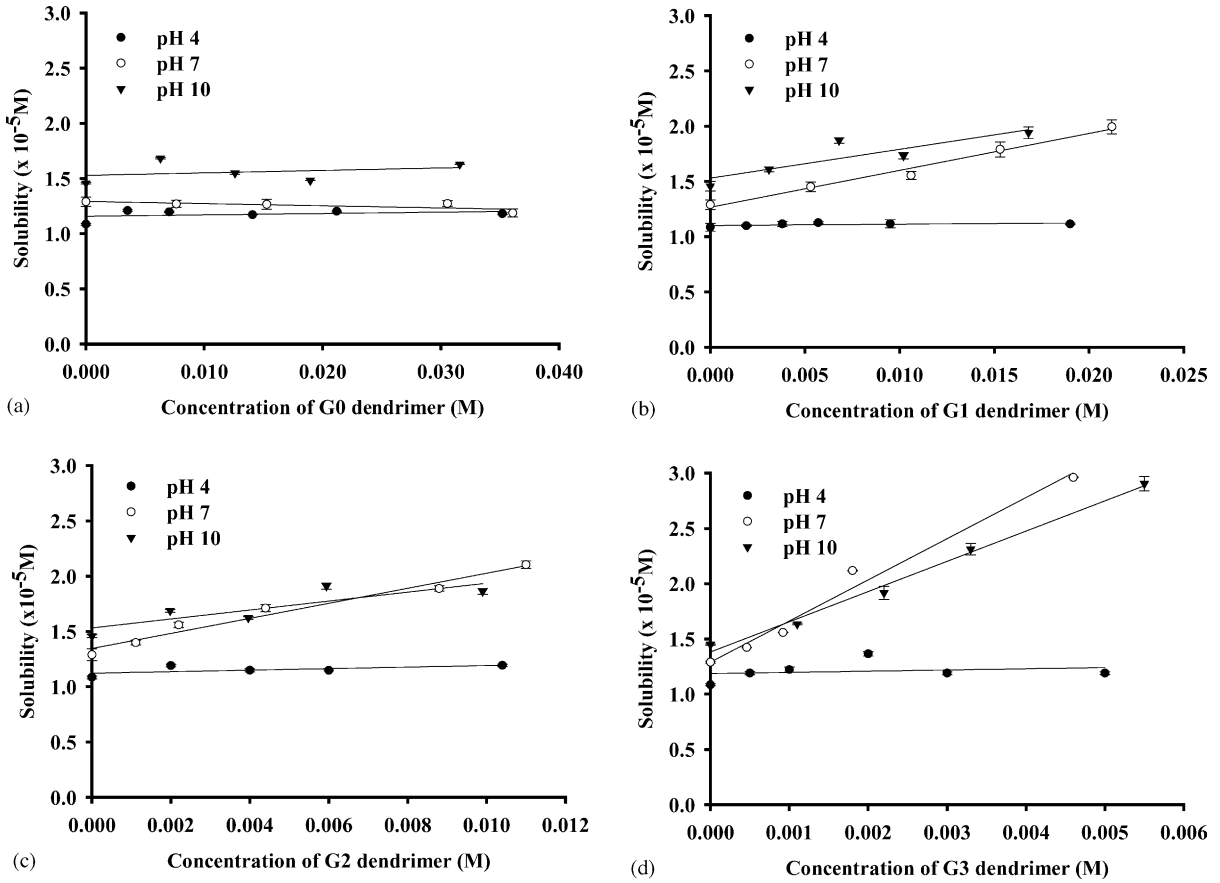


Fig. 2. Solubility profiles of nifedipine in the presence of increasing concentrations of amine-terminated PAMAM dendrimers: (a) G0; (b) G1; (c) G2; and (d) G3.

3.2. Characterization of the nifedipine–dendrimer phase solubility profiles

Solubility profiles of nifedipine measured in the presence of the amine terminated full generation dendrimers at pH 4, 7, and 10 are shown in Fig. 2 and in the presence of ester terminated half-generation dendrimers in Fig. 3. These profiles showed that the G0 dendrimer, Fig. 2, did not increase the aqueous solubility of nifedipine at pH 4, 7, and 10 and the amine terminated full generation dendrimers G1–G3, Fig. 2, did not increase the solubility of nifedipine at pH 4. Statistical analysis of the data suggests that when exceptions mentioned above are excluded, the addition of the dendrimers significantly increased the aqueous solubility of nifedipine at all three pH values.

In the presence of G1–G3 dendrimers at pH 7, Fig. 2, the solubility of nifedipine increased in an approximately linear manner with an increase in dendrimer concentration. A linear increase in nifedipine solubility was also observed at pH 10 for G1–G3 (Fig. 2). In contrast to the amine terminated full generation dendrimers, in the presence of ester terminated half-generation dendrimers, Fig. 3, the aqueous solubility of nifedipine increased linearly with dendrimer concentration at pH 4, 7, and 10. The data in Figs. 2 and 3 also showed that the solubility of nifedipine was highest at pH 7, less at pH 10, and least at pH 4. For a given generation size at pH 7 and 10 the solubility of nifedipine was also greater in the presence of ester terminated dendrimers compared to the amine-terminated dendrimers

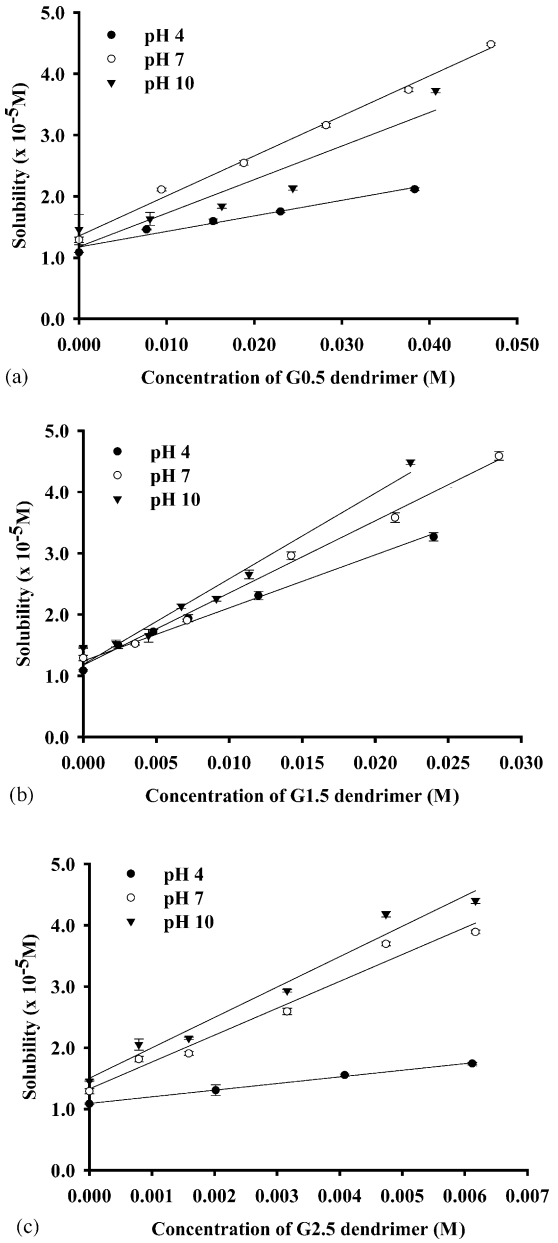


Fig. 3. Solubility profiles of nifedipine in the presence of increasing concentrations of ester-terminated PAMAM dendrimers: (a) G0.5; (b) G1.5; and (c) G2.5.

possessing the same number of surface functional groups.

The solubility profiles where an increase in solubility was observed at pH 7 and 10 for full generation dendrimers, Fig. 2, and for half-generation dendrimers, Fig. 3, were classified as A_L-type diagrams (Higuchi and Conners, 1965). Since the slopes of these profiles are less than unity the soluble complexes between nifedipine and the dendrimers appear to have 1:1 stoichiometries (Table 2). Stability constants calculated from the slopes of the solubility profiles, Table 2, indicated that the amine terminated dendrimers formed very stable complexes at pH 7 compared to pH 10 while for ester-terminated dendrimers the stability constants were approximately equal at pH 7 and 10. Ester terminated dendrimers formed less stable complexes at pH 4. In addition, at a given pH and with the same number of surface functional groups, the ester terminated half-generation dendrimers formed more stable complexes than the amine-terminated dendrimers. The stability constants for dendrimer–nifedipine complexes were found to significantly higher than those for complexes of nifedipine with substituted phenolic ligands (Boje et al., 1988). But the stability constants for the complexes of nifedipine with G0, G1, G2, G0.5, G 1.5 were found to be lower than that for nifedipine-β cyclodextrin 1:1 complex (121.9 M^{-1}) and hydroxypropyl-β-cyclodextrin 1:1 complex (253.7 M^{-1}) where as the

Table 2
Linear regression parameters of the type A_L phase solubility profiles and stability constants for complexes of nifedipine and PAMAM dendrimers

pH	Generation	R^2	$K_{1:1}$ (M^{-1})
4	0.5	0.9689	23.4 ± 1.5
	1.5	0.9683	79.6 ± 2.2
	2.5	0.9892	116.3 ± 3.3
7	1	0.9823	25.6 ± 1.1
	2	0.9675	52.7 ± 2.1
	3	0.9821	287.6 ± 6.1
	0.5	0.8863	42.5 ± 1.3
	1.5	0.9932	91.2 ± 2.5
	2.5	0.9728	338.7 ± 5.6
10	1	0.7593	18.0 ± 1.3
	2	0.7034	27.7 ± 1.6
	3	0.9909	187.1 ± 2.3
	0.5	0.9947	44.8 ± 1.1
	1.5	0.9733	95.7 ± 1.2
	2.5	0.9728	338.5 ± 4.5

$K_{1:1}$ = slope/S_0(1 − slope) where S_0 is the equilibrium solubility of nifedipine in the absence of the dendrimers (Higuchi and Conners, 1965) (n = 3).

stability constants in the presence of G3 and G2.5 at pH 7 and 10 were significantly higher than those for nifedipine cyclodextrin complexes (Chowdary and Reddy, 2002).

3.3. Possible mechanism of interaction between PAMAM dendrimers and nifedipine

Molecular simulations of structures of the PAMAM dendrimers showed that lower generation dendrimers (G < 4 or 4.5) possess an open structure and an ellipsoidal shape, where as later generations (G > 4 or 4.5) are characterized by closed structure with a densily packed surface and a spherical shape (Naylor et al., 1989). In addition, studies have shown that since these lower generation dendrimers are well below deGennes' dense-packing transitions they show very weak pH dependent conformational changes that could interfere with complexation (Chen et al., 2000). Also rheological studies of PAMAM dendrimers under steady shear, creep, and dynamic oscillary shear showed that the lower generation dendrimers showed excellent fit with the predictions of a single-relaxation-mode Maxwell model with the view that with higher generation a qualitative change in molecular conformations may occur where as lower generations are less sensitive to conformational changes (Uppuluri et al., 2000). All this means that the lower generation dendrimers are more accessible for drug inclusion.

Further, PAMAM dendrimers have primary amines on the surface and tertiary amines in their internal cavities, which could act as hydrogen bond donors and acceptors and the nitrogen atom in the dihydropyridine moiety of nifedipine has a covalently bonded hydrogen atom that could act as hydrogen bond donor (Chen et al., 2000). Therefore, nifedipine could interact with the nitrogen atoms of the dendrimer amines by hydrogen bond formation. In addition to hydrogen bonding the solubilization properties of dendrimers could also be due to the hydrophobicity of their microenvironment. Earlier studies have shown that microenvironment in the dendritic microcavities is considerably less polar than the microenvironment of the bulk aqueous phase (Pistolis et al., 1999). Therefore, in aqueous medium, highly hydrophobic nifedipine molecules could be solubilized within the dendrimers at the sites of low polarity (Pistolis et al., 1999). However, at pH 4, protonation of the tertiary amines in the full generation dendrimers creates an environment identical to that of the bulk water inside the dendrimer microcavity and hence no increase in the solubility of nifedipine is observed as seen in Figs. 2 and 3.

Since the number of primary and tertiary amines in the dendrimer increases with generation size (Table 1), at a given pH the protonation state of these amines varies with the generation size and type of the surface functional group. For ethylenediamine core PAMAM dendrimers, primary amines have higher pK_a's (7–9) than the tertiary amines (3–6). Also, tertiary amines closer to the core were found to have a reduced pK_a relative to identical residues closer to the surface (Tomalia et al., 1985; Ottaviani et al., 1996; Chen et al., 2000). These differences in pK_a values and protonation states of the primary and tertiary dendrimer amines correlated with the pH-dependent changes in the aqueous solubility of nifedipine, which is shown in Figs. 2 and 3, and suggest pH dependent hydrogen bond formation between the drug and the dendrimers.

In contrast to the amine terminated full generation dendrimers, in the presence of ester terminated half-generation dendrimers G0.5, G1.5, and G2.5 (Fig. 3) the aqueous solubility of nifedipine increased linearly with dendrimer concentration at all the three pH's. This could be due to reduced protonation of the half-generation dendrimers compared to the full generation dendrimers. This means more unprotonated tertiary amines were available for hydrogen bond formation with the drug molecules (Ottaviani et al., 1996).

These pH dependent changes in the aqueous solubility of nifedipine by dendrimers suggest that the non-covalently bound nifedipine is preferentially bound to the tertiary amines in the dendrimer interior due to the increase in the propensity for the formation of hydrogen bonds with the number of alkyl substitutions on the amine (Taft et al., 1978). In addition, at a specified pH, the larger generations of PAMAM dendrimers possess a less charged internal environment than the smaller generations thereby favoring hydrogen bond formation with the guest molecules.

4. Conclusion

Lower generation (<G3) PAMAM dendrimers have the potential to significantly increase the solubility of poorly water soluble drugs such as nifedipine. The

increase in drug solubility in aqueous PAMAM dendrimer solutions depended on the size of the dendrimer, the type of surface functional groups, and the pH of the medium. For nifedipine the increase in solubility was greatest in the presence of ester terminated half-generation dendrimers at pH 7 because at this pH these dendrimers are less susceptible to protonation compared to the full generation dendrimers. The dependence of nifedipine solubility on pH also offers the possibility to design pH dependent controlled-release drug delivery systems containing the drug trapped inside the dendrimers.

References

Ali, S.L., 1989. Nifedipine. In: Florey, K. (Ed.), Analytical Profiles of Drug Substances, vol. 18. Academic Press, New York, pp. 223–228.

Beezer, A.E., King, A.S.H., Martin, I.K., Mitchel, J.C., Twyman, L.J., Wain, C.F., 2003. Dendrimers as potential drug carriers; encapsulation of acidic hydrophobes within water soluble PAMAM derivatives. Tetrahedron 59, 3873–3880.

Boje, K.M., Sak, M., Fung, H.L., 1988. Complexation of nifedipine with substituted phenolic ligands. Pharm. Res. 5, 655–659.

Chauhan, A.S., Sredevi, S., Chalasani, K.B., Jain, A.K., Jian, S.K., Jain, N.K., Diwan, P.V., 2003. Dendrimer-mediated transdermal delivery: enhanced bioavailability of indomethacin. J. Control. Rel. 90, 335–343.

Chen, W., Tomalia, D.A., Thomas, L.J., 2000. Unusual pH-dependent polarity changes in PAMAM dendrimers: Evidence for pH-responsive conformational changes. Macromolecules 33, 9169–9172.

Chowdary, K.P.R., Reddy, G.K., 2002. Complexes of nifedipine with β- and hydroxypropyl β-cyclodextrins in the design of nifedipine SR tablets. Ind. J. Pharm. Sci. 64, 142–146.

De Muth, J.E., 1999. Basic Statistics and Pharmaceutical Statistical Applications. Marcel Dekker, New York, USA.

Emara, L.H., Badr, R.M., Abd Elbary, A., 2002. Improving the dissolution and bioavailability of nifedipine using solid dispersions and solubilizers. Drug Dev. Ind. Pharm. 28, 795–807.

El-Sayed, M., Ginski, M., Rhodes, C., Ghandehari, H., 2002. Transepithelial transport of poly(amidoamine) dendrimers across Caco-2 cell monolayers. J. Control Rel. 81, 355–365.

Esfand, R., Tomalia, D.A., 2001. Dendrimers and Other Dendritic Polymers. John Wiley, New York, pp. 589–604.

Frechet, J.M.J., 1994. Functional polymers and dendrimers: reactivity, molecular architecture, and interfacial energy. Science 263, 1710–1715.

Higuchi, T., Conners, K.A., 1965. Phase-solubility techniques. In: Reilly, C.N. (Ed.), Advances in Analytical Chemistry and Instrumentation. John Wiley, New York, pp. 117–212.

Kleinbloesem, C.H., Van Harten, J., De Leede, L.J.G., Van Brummelen, P., Breimer, D.D., 1984. Nifedipine: kinetics and dynamics in healthy subjects. Clin. Pharmacol. Ther. 36, 396–401.

Kojima, C., Kono, K., Maryama, K., Tagagishi, T., 2000. Synthesis of polyamidoamine dendrimers having poly (ethylene) glycol grafts and their ability to encapsulate anticancer drugs. Bioconjugate Chem. 11, 910–917.

Kolhe, P., Misra, E., Kannan, R.M., Kanna, S., Lieh-Lai, M., 2003. Drug complexation, in vitro release and cellular entry of dendrimers and hyperbranched polymers. Int. J. Pharm. 259, 143–160.

Naylor, A.M., Goddard III, W.A., Kiefer, G.E., Tomalia, D.A., 1989. Starburst dendrimers 5: molecular shape control. J. Am. Chem. Soc. 111, 2339–2341.

Ottaviani, M.F., Montalti, F., Romanelli, M., Turro, N.J., Tomalia, D.A., 1996. Characterization of starburst dendrimers by EPR. 4. Mn(II) as a probe of interphase properties. J. Phys. Chem. 100, 11033–11042.

Patri, A.K., Majoros, I.J., Baker, J.R., 2002. Dendritic polymer macromolecular carriers for drug delivery. Cur. Opin. Chem. Biol. 6, 466–471.

Pistolis, G., Milliaris, A., Tsiourvas, D., Paleos, C.M., 1999. Poly (propyleneimine) dendrimers as pH-sensitive controlled release systems. Chem. Eur. J. 5, 1440–1445.

Poxon, S.W., Mitchell, P.M., Liang, E., Hughes, J.A., 1996. Dendrimer delivery of oligonucleotides. Drug Del. 3, 255–261.

Taft, R.W., Wolf, F.J., Beauchamp, J.L., Scorrano, G., Arnette, E.M., 1978. Solvent effect of water and fluorosulfuric acid on proton transfer equilibria and the energies of salvation of gaseous onium ions. J. Am. Chem. Soc. 100, 1240–1249.

The United States Pharmacopeia XXIV, 2000. The United State Pharmacopeial Convention Inc., Rockville, MD.

Tomalia, D.A., Baker, H., Dewald, J., Hall, M., Kallos, G., Martin, S., Roeck, J., Ryder, J., Smith, P., 1985. A new class of polymers: starbust-dendrimtic macromolecules. Polym. J. 17, 117–132.

Twyman, L.J., Beezer, A.E., Esfand, R., Hardy, M.J., Mitchell, J.C., 1999. The synthesis of water soluble dendrimers, and their application as possible drug delivery systems. Tetrahedron Lett. 40, 1743–1746.

Uppuluri, S., Morrison, F.A., Dvornic, P.R., 2000. Rheology of dendrimers. 2. Bulk polyamidoamine dendrimers under steady shear, creep, and dynamic oscillatory shear. Macromolecules 33, 2551–2560.

Vippagunta, S.R., Maul, K.A., Tallavajhala, S., Grant, D.J.W., 2002. Solid-state characterization of nifedipine solid dispersions. Int. J. Pharm. 236, 111–123.

Wiwattanapatapee, R., Carreno-Gomez, B., Malik, N., Duncan, R., 2000. Anionic PAMAM dendrimers rapidly cross adult rat intestine in vitro: a potential oral delivery system? Pharm. Res. 17, 991–997.

Wiwattanapatapee, R., Jee, R.D., Duncan, R., 1999. PAMAM dendrimers as potential oral drug delivery systems: dendrimer complexes with piroxicam. Proc. Int. Symp. Control. Rel. Bioact. Mater. 26, 241.

Yalkowsky, S.H., 1999. Solubility and Solubilization in Aqueous Media. Oxford University Press, New York, USA.