Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Medicinal Chemistry 40 (2005) 1384–1389
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
www.elsevier.com/locate/ejmech

Preliminary communication

Solubility of nicotinic acid in polyamidoamine dendrimer solutions

Cheng Yiyun [a,*], Xu Tongwen [b]

[a] *Laboratory of Structural Biology, Department of Polymer Science and Engineering, School of Life Science, University of Science and Technology of China (USTC), Hefei, Anhui 230027, China*
[b] *Department of Chemistry, University of Science and Technology of China, Hefei, Anhui 230026, China*

Received 13 July 2005; accepted 2 August 2005

Available online 13 October 2005

Abstract

In the present study we investigated the effect of ethylenediamine (EDA) core polyamidoamine (PAMAM) dendrimers on the aqueous solubility of nicotinic acid. The aqueous solubility of nicotinic acid was measured in the presence of dendrimers at room temperature in distilled water. The effect of variables, such as pH condition, concentration, surface functional group and generation (molecule size) of dendrimer, has been investigated. Results showed that the solubility of nicotinic acid in the dendrimer solutions was proportional to dendrimer concentration, both amine and ester-terminated dendrimers caused the higher increase in nicotinic acid solubility at higher pH conditions. The order in which the dendrimers increased the solubility at a constant pH condition was G4 > G3 > G2 > G1. In addition, at each pH, the solubility of nicotinic acid was greater in the presence of amine-terminated dendrimers compared to the amine ester-terminated dendrimers possessing the same number of surface functional groups. Under suitable conditions PAMAM dendrimers can be highly effective used to enhance the solubility of nicotinic acid.

Keywords: Polyamidoamine dendrimers; PAMAM; Solubility enhancer; Nicotinic acid

1. Introduction

Dendrimers, artificial polymers topologically based on the structure of a tree, are synthetic, highly branched, spherical, mono-disperse macromolecules. They combine typical characteristics of small organic molecules and polymers that results in special physical and chemical properties [1–4]. Polyamidoamine (PAMAM) with an ellipsoidal or spheroidal shape is one of the most-studied starburst macromolecules. Due to specific synthesis PAMAM dendrimers have some interesting properties, which distinguish them from classical linear polymers, e.g. PAMAM has a much higher amino group density comparing with conventional macromolecules, a third generation PAMAM prepared from ammonia core has 1.24×10^{-4} amine moieties per unit volume (cubic Angstrom units) in contrast to the 1.58×10^{-6} amine moieties per unit volume of a conventional star polymer [4]; Also, PAMAM Dendrimers possess empty internal cavities and many functional end groups which are responsible for high solubility and reactivity. These specific properties make dendrimers suitable for drug delivery systems [5–7]. Drugs or other molecules can either be attached to dendrimers end groups or encapsulated in the macromolecule interior [8]. The high density of amino groups and special structure in PAMAM dendrimers may be expected to have potential applications in enhancing the solubility of the low aqueous solubility drugs and as delivery systems for bioactive materials [9]. This study uses PAMAM dendrimers (G1-G4) to investigate the potential of PAMAM dendrimers to increase the solubility of drugs as exemplified by nicotinic acid.

Nicotinic acid, 3-carboxylic pyridine, the deficiency of which leads to Pellagra, is an essential dietary vitamin. It is required for cell respiration, helps in the release of energy and metabolism of carbohydrates, fats and proteins, proper circulation and healthy skin, functioning of the nervous system and normal secretion of bile and stomach fluids. It is also used in the synthesis of sex hormones, treating schizophrenia and other mental illnesses, and a memory-enhancer [10]. The physiologically active form of nicotinic acid is nicotinamide adenine dinucleotide (NAD) or its phosphate (NADP). Both NAD and NADP function as coenzymes for a wide variety of

* Corresponding author. Tel./fax: +86 55 1360 3913.
E-mail address: ycheng@mail.ustc.edu.cn (C. Yiyun).

0223-5234/$ - see front matter © 2005 Elsevier SAS. All rights reserved.
doi:10.1016/j.ejmech.2005.08.001

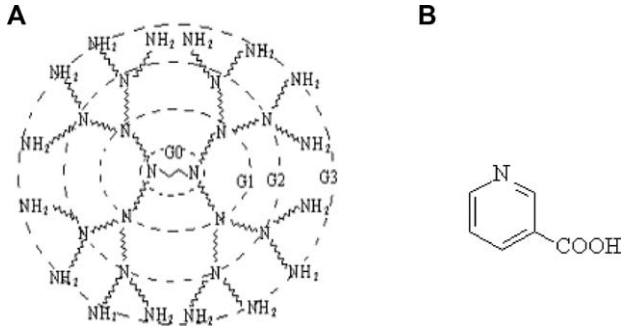

Fig. 1. Schematically molecular structures of G3 PAMAM dendrimer (A) and nicotinic acid (B).

proteins that catalyze oxidation–reduction reactions essential for tissue respiration. However, it is not freely soluble in water at room temperature, so we choose this important and common drug for an investigation.

The aim of the present work was (1) to study the potential of PAMAM dendrimers as a solubility enhancer of the mentioned drug nicotinic acid; (2) to study the effect of pH, concentration, surface functional group and generation on interactions between PAMAM dendrimers and nicotinic acid (Fig. 1).

2. Experiments

2.1. Materials

Nicotinic acid was purchased from Weifang Sunwin Chemicals Co., Ltd (Shangdong, China). Ethylenediamine, methyl acrylate, methanol (HPLC grade) were obtained from Shanghai Chemical Co. (Shanghai, China). For both solubility studies, water used to prepare solutions was two-distilled water.

2.2. Synthesis of PAMAM dendrimers

PAMAM dendrimers were synthesized by the following method (Fig. 2) [2,4]. Ethylenediamine (10.0 g, 0.166 mol) was dissolved in 100 ml methanol in a 1-l round-bottomed flask. Methyl acrylate (94.6 g, 0.751 mol) was added at 40 °C and the system stirred for 24 h under nitrogen. Excess methyl acrylate was removed under vacuum at room temperature. A Michael addition between the amine and the acrylate yielded a product bearing four terminal methyl ester groups, defined as the G0.5 PAMAM. Subsequently, ethylenediamine (120 g, 2.00 mol) was dissolved in methanol and added to the G0.5 PAMAM and, after stirring for 48 h under nitrogen and removing excess reactants by vacuum distillation, a product bearing four terminal amino groups were obtained, defined as the G1 PAMAM. By repeating the above cycle, higher generation PAMAM dendrimers (up to G4) were synthesized. Since the dendrimers are highly hygroscopic, they were stored as 10% (w/w) solutions in methanol. The characteristic data of different generations of PAMAM are listed in Table 1.

2.3. Solubility testing experiments

The solubility of nicotinic acid in PAMAM dendrimer solutions in the range 0–10 mg/ml was determined at pH 3, 4 and 6 in phosphate buffers (0.05 M NaH_2PO_4). The method used

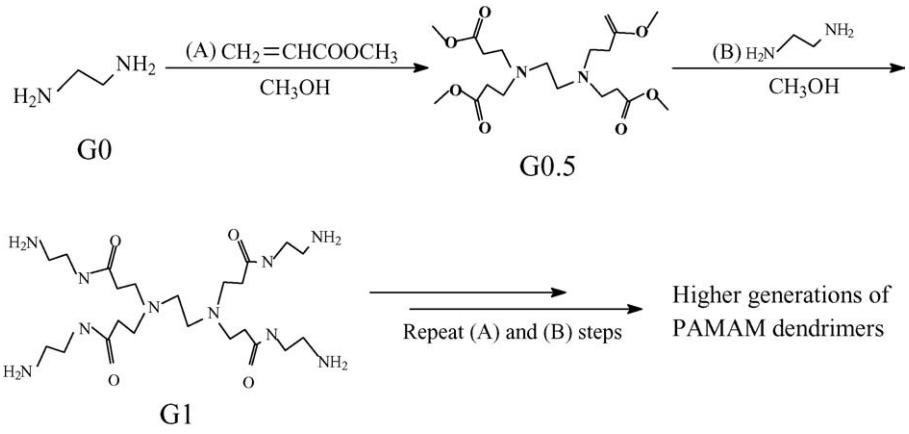

Fig. 2. Synthesis strategy for PAMAM dendrimers.

Table 1
The characteristic data of PAMAM dendrimers

Generation	Molecular formula	Molecular weight	Number of terminal amino/ester groups	Number of total amino groups	Radius from SAXS (Å)
G1	$C_{22}H_{48}O_4N_{10}$	516	4	10	–
G2	$C_{62}H_{128}O_{12}N_{26}$	1428	8	26	–
G3	$C_{142}H_{288}O_{28}N_{58}$	3252	16	58	15.8
G4	$C_{302}H_{608}O_{60}N_{122}$	6900	32	122	17.1
G5	$C_{622}H_{1248}O_{124}N_{250}$	14196	64	250	24.1

for sample preparation was similar for each system, i.e. excess nicotinic was added to 5 ml vials containing 4 ml of each test solution. The vials were then incubated in a shaking water bath at 37 °C for 24 h. Then the solutions were centrifuged at 5000 rpm for a minute and the absorbance of the nicotinic acid test solutions at the characteristic wavelength 262 nm were tested using the Varian Cary VIII spectrophotometer. Three repeats were conducted for each sample.

3. Results and discussion

3.1. Structural characterization of the PAMAM dendrimers

Hydrodynamic diameters of ester-terminated PAMAM dendrimers (G 0.5, G 1.5, G2.5, and G3.5) in ethyl acetate solution were determined by dynamic light scattering method (DYNAPRO-99, USA). For sample preparation, 1–2 wt% PAMAM/ethyl acetate solution was repeatedly filtrated through a 0.1 um nylon filter. Purity of the amine-terminated PAMAM dendrimers were characterized via FT-IR (MAGNA-IR 750, Nicolet Instrument Co., U.S.A), ^{1}H and ^{13}C NMR (DMX-500, German), Mass spectral analysis (BIFI EXTM 3, German) and Element analysis (VARIO EL 3, Elementar Instrument Co., German), and the results agreed with that reported in the literature [2,4]. Since the dendrimers are highly hygroscopic, they were stored as 10% (w/w) solutions in methanol.

3.2. Effect of PAMAM concentration on solubility of nicotinic acid

The effect PAMAM dendrimer concentration on solubility of nicotinic acid was carried out using G3 PAMAM dendrimer of molecular weight 3252 Da and 16 amine groups in the outer shell, and the results were shown in Fig. 3. It was observed that the solubility of nicotinic acid increased significantly with PAMAM concentrations. In the presence of G3 PAMAM dendrimer at a fixed pH condition, the solubility of nicotinic acid in the dendrimer solutions increased in an approximately linear manner with an increase in dendrimer concentration. This was presumably due to the increase in the number of surface amines and internal cavities that are available to interact with nicotinic acid molecules. 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 density packed surface and a spherical shape [11]. Due to this specific and interesting property of PAMAM dendrimers, the cavities in PAMAM dendrimers can keep small guest molecules inside and make dendrimers suitable for enhancing the solubility of drug molecules such as nicotinic acid in aqueous solutions. Also, there are tertiary amines in these internal cavities, which could interact with the atoms of the nicotinic acid molecules by hydrogen bond formation. Further, PAMAM dendrimers have primary amines on the surface, which could interact electrostatically with the carboxyl group in the nicotinic acid molecules. Therefore, G3 PAMAM dendrimers possess open and internal cavities and many functional terminal groups that are responsible for high solubility and reactivity. These specific properties make dendrimers suitable for drug delivery systems [5].

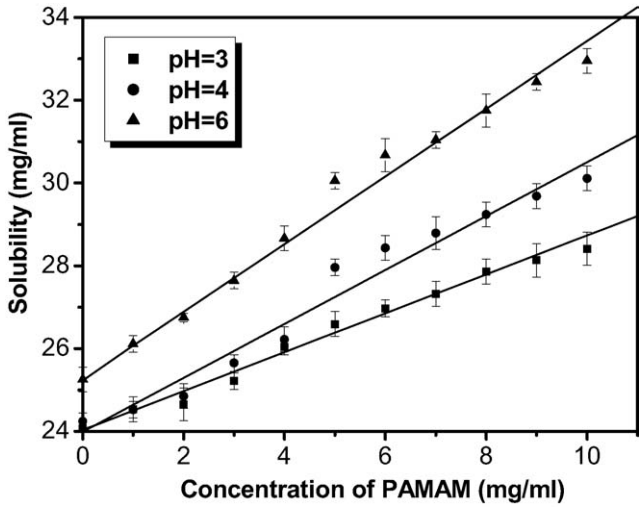

Fig. 3. Solubility of nicotinic acid in the presence of increasing concentration of G3 PAMAM dendrimer.

3.3. Effect of pH condition on solubility of nicotinic acid

To ascertain the most effective pH condition on solubility of nicotinic acid using PAMAM dendrimers, samples of nicotinic acid solution were produced at a range of pH values, the concentration of G3 PAMAM dendrimer being constant. The results are shown in Fig. 3 and, as can be seen, the process was pH-dependent. The solubility of nicotinic acid in PAMAM dendrimer solutions was highest at pH 6, less at pH 4, and least at pH 3. Studies have shown that lower generation dendrimers are very weak pH-dependent conformational changes [12]. Also rheological studies of PAMAM dendrimers showed that the lower generation dendrimers showed excellent fit 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 [13]. All these mean that the lower generation dendrimers are more accessible for drug inclusion. As the PAMAM dendrimers used in this study was lower generation ones (G1–G4), we can presume that the conformation of PAMAM dendrimers did not change at various pH conditions. As discussed in 3.2, the solubility enhancement of nicotinic acid is due to an electrostatically interaction between the surface amine groups of dendrimer molecule and the carboxyl group of nicotinic acid and to hydrogen bond formations between tertiary amines in internal cavities of dendrimers and the atoms of nicotinic acid (Fig. 4). At lower pH conditions, there is a lower increase of solubility of nicotinic acid in dendrimer solution compared to that at higher pH con-

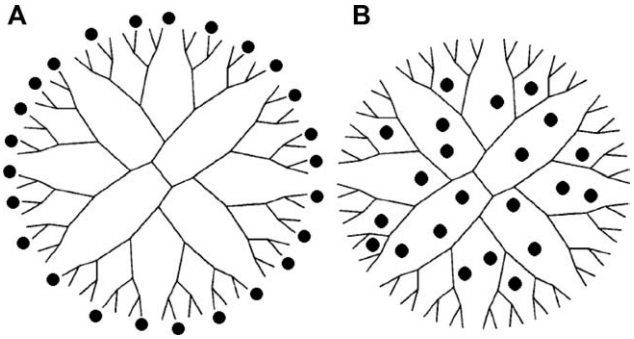

Fig. 4. Two potential strategies for interactions between PAMAM dendrimers and nicotinic acid molecules.

ditions. This is because the nicotinic acid, being weakly acid, is not fully ionized at low pH conditions and hence cannot freely interact electrostatically with the surface amine groups of dendrimer molecule. Further, Earlier studies have shown that the cavity environment inside the PAMAM dendrimer is more hydrophobicity than that of the water phase outside [14]. Therefore, in PAMAM dendrimer solutions, nicotinic acid molecules could be solubilized in the cavities of the PAMAM dendrimer at the site of low polarity [14]. However, at low pH conditions, protonation of the tertiary amines in the full generation dendrimers enhances the polar level of environment inside the dendrimer, which causes no significant increase in the solubility of nicotinic acid as observed in the experiment (Fig. 3). As a result, the solubility of nicotinic acid in PAMAM dendrimer solutions was lowest at pH 3.

3.4. Effect of surface functional group of PAMAM on solubility of nicotinic acid

The effect of surface functional group of PAMAM dendrimers (amine-terminated and ester-terminated) on the process was investigated at pH 4. The G3 PAMAM dendrimer, used in the present paper, possesses 16 amino groups on a surface whereas PAMAM G2.5 dendrimers possess 16 carboxylate groups on the surface. The results are shown in Fig. 5. In contrast to the amine-terminated full generation dendrimers, in the presence of ester-terminated G2.5 PAMAM dendrimers, the aqueous solubility of nicotinic acid also increased linearly with dendrimer concentration at pH 4. It is clear that the solubility of nicotinic acid was affected by the surface functional group of PAMAM dendrimer. The solubility of nicotinic acid in amine-terminated dendrimer solution was obviously higher that those in ester-terminated ones. This is because that the solubility of nicotinic acid in amine-terminated PAMAM solutions depends on the primary amines on the surface and cavities inside the PAMAM particles, while the solubility in ester-terminated PAMAM solutions depends on cavities inside the PAMAM particles. Thus the amine-terminated PAMAM dendrimers have a higher ability to enhance the solubility of nicotinic than ester-terminated PAMAM dendrimers.

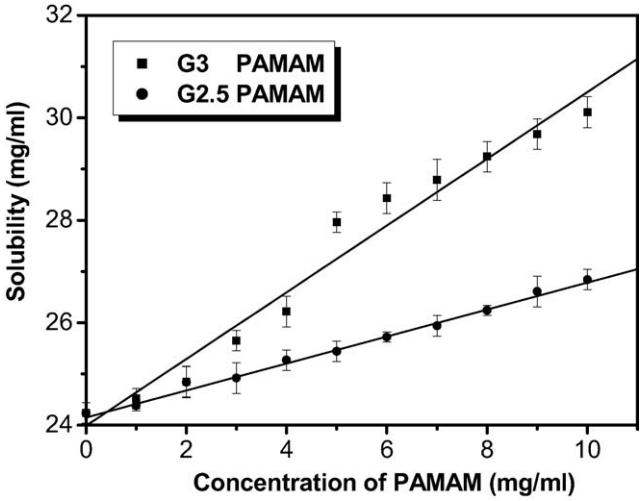

Fig. 5. Solubility of nicotinic acid in the presence of increasing concentration of G2.5 and G3 PAMAM dendrimer at pH 4.

3.5. Effect of generation of PAMAM on solubility of nicotinic acid

The effect of various generations of PAMAM dendrimers (G1–G4) on the process was investigated at pH 3, 4 and 6, respectively. The results are shown in Figs. 6–9, from which it is clear that the solubility of nicotinic acid was affected by the generation of PAMAM dendrimer. The solubility of nicotinic acid in higher generation PAMAM solution was in fact higher that those in lower ones. The solubility of hydrophobic compounds in dendrimer solutions likely depends on the dendrimer generation (size) [15]. Since the number of primary and tertiary amines in the dendrimer increases with generation size, at a given pH condition, higher generation dendrimer has a tendency to entrap more hydrophobic compound inside than lower ones. Also, the solubility of nicotinic acid in PAMAM solutions depends on the surface area and primary amino groups of PAMAM particles, which cause the higher generation PAMAM particles to have a higher ability

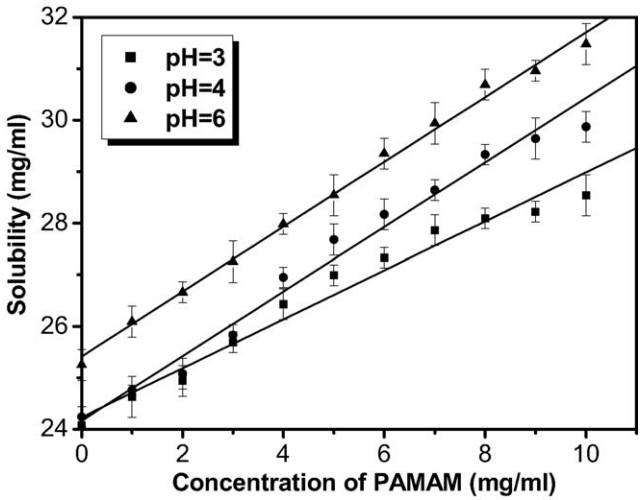

Fig. 6. Solubility of nicotinic acid in the presence of increasing concentration of G1 PAMAM dendrimer.

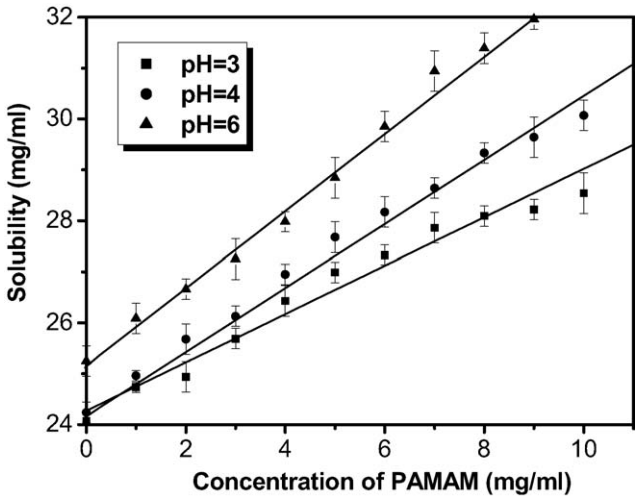

Fig. 7. Solubility of nicotinic acid in the presence of increasing concentration of G2 PAMAM dendrimer.

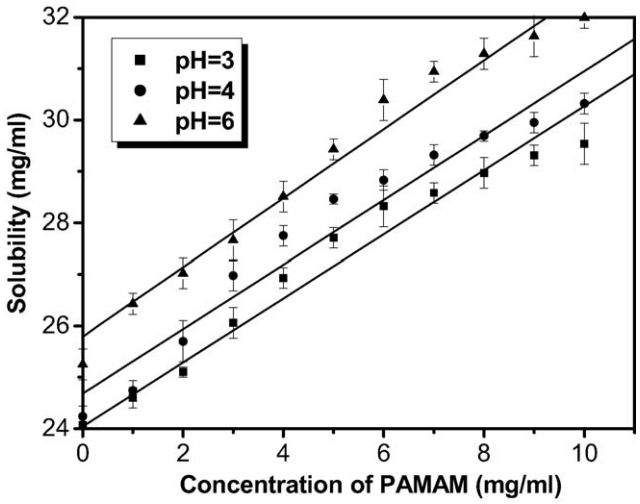

Fig. 8. Solubility of nicotinic acid in the presence of increasing concentration of G4 PAMAM dendrimer.

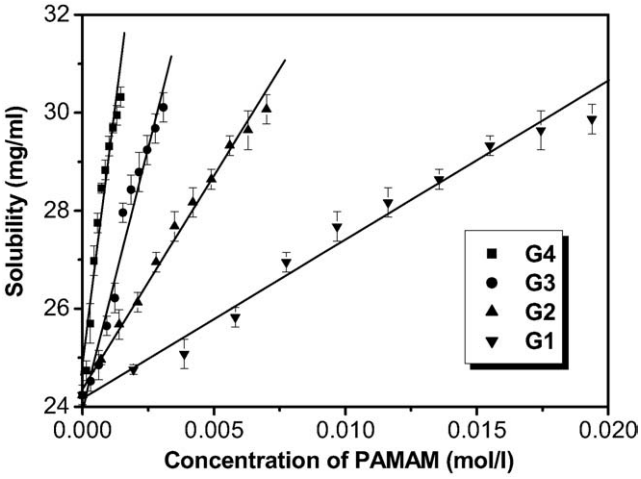

Fig. 9. Solubility of nicotinic acid in the presence of increasing concentration of G1, G2, G3 and G4 PAMAM dendrimer at pH 4.

to absorb and interact with the nicotinic acid molecule. In this way, we could explain why higher generation dendrimers could enhance the solubility of nicotinic acid more efficiently than lower ones.

4. Conclusion

Different generation (G1–G4) PAMAM dendrimers have the potential to significantly enhance the solubility of nicotinic acid. The drug solubility depends on the concentration of the dendrimer, the pH value of the solution, and surface functional group and the generation of the dendrimer. Solubility of nicotinic acid in the dendrimer solutions increase in an approximately linear manner with an increase in dendrimer concentration; Both amine and ester-terminated dendrimers cause the higher increase in nicotinic acid solubility at higher pH conditions; The order in which the dendrimers increased the solubility at a constant pH condition is G4 > G3 > G2> G1; In addition, at each pH, the solubility of nicotinic acid is greater in the presence of amine-terminated dendrimers compared to the amine ester-terminated dendrimers possessing the same number of surface functional groups. Both observations are evidence of the solubility enhancement of nicotinic acid is due to an electrostatically interaction between the surface amine groups of dendrimer molecule and the carboxyl group of nicotinic acid and to hydrogen bond formations between tertiary amines in internal cavities of dendrimers and the atoms of nicotinic acid.

Acknowledgements

Financial supports from Innovation Foundation of Graduate Student in University of Science and Technology of China (KD2004035) were highly appreciated.

References

[1] D.A. Tomalia, H. Baker, J. Dewald, A new class of polymers: starburst-dendritic macromolecules, Polym. J. 17 (1985) 117.
[2] D.A. Tomalia, H. Baker, J.R. Dewald, Dendritic molecules: synthesis of starburst dendrimer, Macromolecules 19 (1986) 2466.
[3] D.A. Tomalia, J.R. Dewald, (1986) Dense star polymers having two-dimensional molecular diameter. U.S. Patent 4587329.
[4] D.A. Tomalia, A.M. Naylor, W.A. Goddard, Starburst dendrimers: molecular-level control of size, shape, surface chemistry, topology, and flexibility from atoms to macroscopic matter, Angew. Chem. Int. Ed. Engl. 29 (1990) 138.
[5] E. Roseita, D.A. Tomalia, Poly (amidoamine) (PAMAM) dendrimers: from biomimicry to drug delivery and biomedical applications, Drug Delivery Today 6 (2001) 427–436.
[6] O. Schiavon, G. Pasut, S. Moro, PEG–Ara–C conjugates for controlled release, Eur. J. Med. Chem. 39 (2004) 123–133.
[7] M.F. Brana, G. Dominguez, B. Saez, Synthesis and anti-tumor activity of new dendritic polyamines–(imide–DNA–intercalator) conjugates: potent Lck inhibitors, Eur. J. Med. Chem. 37 (2002) 541–551.

[8] P. Furuta, J.M. Frechet, Controlling solubility and modulating peripheral function in dendrimer encapsulated dyes, J. Am. Chem. Soc. 125 (2003) 13173–13181.
[9] G.R. Newkome, C.N. Moorefield, G.R. Baker, Alkane cascade polymers processing micellar topology: micellanoic acid derivatives, Angew. Chem. Int. Ed. Engl. 30 (1991) 1178–1180.
[10] C.T. Supuran, A. Scozzafava, L. Menabuoni, Carbonic anhydrase inhibitors—part 70. Synthesis and ocular pharmacology of a new class of water-soluble, topically effective intraocular pressure lowering agents derived from nicotinic acid and aromatic/heterocyclic sulfonamides, Eur. J. Med. Chem. 34 (1999) 799–808.
[11] A.M. Naylor, I.I.I. Goddard, D.A. Tomalia, Starburst dendrimers 5: molecular shape control, J. Am. Chem. Soc. 111 (1989) 2339–2341.
[12] W. Chen, D.A. Tomalia, L.J. Thomas, Unusual pH-dependent polarity changes in PAMAM dendrimers: Evidence for pH-responsive conformational changes, Macromolecules 33 (2000) 9169–9172.
[13] S. Uppuluri, F.A. Morrison, P.R. Dvornic, Rheology of dendrimers. 2. Bulk polyamidoamine dendrimers under steady shear, creep, and dynamic oscillatory shear, Macromolecules 33 (2000) 2551–2560.
[14] G. Postolis, A. Milliaris, D. Tsiourvas, Polyamidoamine dendrimers as pH-sensitive controlled release systems, Chem. Eur. J. 5 (1999) 1440–1445.
[15] K. Chie, K. Kenji, M. Kazuo, Synthesis of polyamidoamine dendrimers having poly (ethylene glycol) grafts and their ability to encapsulate anticancer drugs, Bioconjugate Chem. 17 (2000) 910–917.