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European Journal of Medicinal Chemistry 43 (2008) 1791–1795
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
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Laboratory note

Potential of poly(amidoamine) dendrimers as drug carriers of camptothecin based on encapsulation studies

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

[a] *Department of Chemistry, University of Science and Technology of China, Hefei, Anhui 230026, PR China*
[b] *Hefei National Laboratory for Physical Sciences at Microscale and School of Life Sciences, University of Science and Technology of China, Hefei, Anhui 230027, PR China*

Received 1 September 2007; received in revised form 24 September 2007; accepted 24 September 2007
Available online 22 January 2008

Abstract

Camptothecin (CPT), a plant alkaloid isolated from *Camptotheca acuminata*, has an extremely low solubility in aqueous medium, which presents a major challenge during drug formulation in clinical trails. In the present study we investigated the potential of poly(amidoamine) (PAMAM) dendrimers as drug carriers of CPT through aqueous solubility studies. Results showed that the aqueous solubility of CPT was significantly increased by PAMAM dendrimers. The effect of PAMAM generation on CPT solubility was also evaluated. These studies indicated that PAMAM dendrimers might be considered as biocompatible carriers of CPT.

Keywords: Poly(amidoamine) dendrimers; PAMAM; Drug carrier; Anti-cancer drugs; Camptothecin; Solubility

1. Introduction

Camptothecin (CPT), a plant alkaloid isolated from *Camptotheca acuminata*, is a potent, anti-cancer agent against a wide spectrum of human cancers, including lung, prostate, breast, colon, stomach, bladder, ovarian and melanoma cancers [1]. Unlike many other anti-cancer drugs, which inhibit cancer cell proliferation by binding the DNA, CPT acts by binding to the DNA replicating enzyme topoisomerase I and destroying DNA strands during DNA replication in the cell cycle, finally causing cell death if the broken DNA is not repaired [2].

Since CPT was first isolated in 1966, it has already gained a great deal of interest because of its excellent *in vitro* and *in vivo* anti-tumor activities. But in spite of promising results obtained during cell and animal studies, clinical trials of CPT were abandoned due to (1) the extremely low solubility in water and (2) unexpected toxicity [2]. Poor solubility restricts its use in topical and parenteral trials and is generally related to a low bioavailability, presenting a major challenge during drug formulation.

To overcome the solubility problem of CPT, it is usually suggested that water-soluble CPT analogs be synthesized or novel delivery systems be developed for effective administration of CPT [3]. Water-soluble CPT analogs have increased solubility but decreased potency in anti-tumor activity compared with CPT. Therefore, the development of novel delivery systems is attracting more and more attention. Liposomes, cyclodextrins, microspheres, microemulsions and other polymers have already been used to prepare new CPT formations/complexes [4,5,25,32–37]. However, these CPT formations have been limited in clinical trails, as they don't exhibit effective anti-cancer activities when they were orally or intravenously administrated [5]. Development of new drug delivery system (DDS) for CPT is still one of the most important goals in cancer chemotherapy today.

Dendrimers are new-artificial macromolecules topologically based on the structure of a tree. They are hyperbranched,

* Corresponding author. Department of Chemistry, University of Science and Technology of China, Hefei, Anhui 230026, PR China.
** Corresponding author.
E-mail addresses: yycheng@mail.ustc.edu.cn (Y. Cheng), twxu@ustc.edu.cn (T. Xu).

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doi:10.1016/j.ejmech.2007.09.030

monodisperse, three-dimensional molecules, having defined theoretical molecular weight and host–guest entrapment properties. Due to their special synthesis in a step-wise manner from branched monomer units, they allow the precise control of size, shape, dimensions, density, polarity, flexibility, solubility and placement of functional groups by choosing these building units and functional group chemistry. As a result, they combine typical characteristics of small organic molecules and polymers that result in special physical and chemical properties [6–9]. Up to now, dendrimers have already attracted increasing attention for their applications in many fields including host–guest chemistry or combination chemistry, electrochemistry and photochemistry, nanoparticle synthesis template, water purification, dye decolorization, monomolecular membranes, curing agents in epoxy resin systems, catalyzer in extensive areas, drug delivery systems and gene transfection in biomedical fields. Among them the use of dendrimers as drug carriers in delivery systems has been of great interest.

Poly(amidoamine) (PAMAM) dendrimers with ellipsoidal or spheroidal shapes are 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 compared 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 [6,7]. The high density of functional groups ($-NH_2$, $-COOH$, $-OH$) in PAMAM dendrimers may be expected to have potential applications in enhancing the solubility of low aqueous solubility drugs and delivery systems for bioactive materials. Also, these functional groups on the outer shell are responsible for high reactivity which means dendrimers can be modified or conjugated with a list of interesting guest molecules. Furthermore, PAMAM dendrimers possess empty internal cavities, which are able to encapsulate hydrophobic guest molecules in the macromolecule interior. Drugs or other molecules can either be attached to dendrimers' end groups or encapsulated in the macromolecule interior [10]. These specific properties make dendrimers suitable for drug delivery systems [11–14]. Drugs bound to dendrimers are at early stages of development and data on them are limited. Several authors reported on the solubilization of non-steroidal anti-inflammatory drugs (NSAIDs) [15–19], anti-bacterial agents (sulfonamides and quinolones) [20,21] and anti-cancer drugs by dendrimers [22,23]. Here, we focus on using PAMAM dendrimers with an ethylenediamine (EDA) core as potential drug carriers of CPT.

2. Experiments

2.1. Materials

G4, G5, G6 PAMAM dendrimers used in this present study were purchased from Sigma–Aldrich Co. (U.S.A.). CPT was received as a gift from Prof. You Tianpa from the Department of Chemistry, University of Science and Technology of China; methanol, dimethylsulfoxide (DMSO) and acetonitrile (HPLC grade) were purchased from Tedia Company (U.S.A.). For both solubility studies and HPLC assay, deionized water was used.

2.2. Solubility test

The solubility of CPT was determined using the equilibrium solubility method [24] as follows. Excess drugs were added to 500 μl of each test solution (G4, G5 and G6) to ensure the drug solution reaching saturation. The solution was mechanically shaken for 24 h at 37 °C and then the solutions were centrifuged at 10,000 rpm for 10 min. Three repeats were conducted for each sample. The saturated solutions obtained from the solubility studies were diluted to a proper concentration with deionized water (400× for CPT solubility in dendrimer solutions and 5× for CPT solubility in deionized water). The diluted samples were then analyzed by high performance liquid chromatography (HPLC).

2.3. HPLC analysis

The amount of CPT in the samples obtained in aqueous solubility studies was estimated by reversed phase high performance liquid chromatography (RT-HPLC) (Agilent, Germany) with a reversed phase C_{18} column (5 μm, 250 mm × 5 mm) maintained at room temperature. Briefly, the detector was set at an excitation wavelength of 370 nm and an emission wavelength of 435 nm using a mobile phase of acetonitrile: aqueous triethylamine–acetate buffer (prepared using 0.1% v/v triethylamine, adjusted with glacial acetic acid to pH 5.5) = 27:73 delivered at a flow rate of 1.0 ml/min. The injection volume used was 20 μl. As CPT usually exists in two forms, a lactone form (CPT-lactone) and a carboxylate form (CPT-carboxylate). The standard solutions of CPT-lactone and CPT-carboxylate were made by dilution of CPT stock solution in DMSO with 0.02 M HCl and 0.02 M NaOH solutions, respectively. The analysis of CPT-carboxylate was carried out 2 h after preparing the standard solution to ensure the complete conversion of CPT-lactone to CPT-carboxylate [25]. The calibration curves of CPT-carboxylate and CPT-lactone were $y = 1.50 \times 10^6 x - 21$ ($r^2 = 0.9989$) and $y = 1.80 \times 10^6 x + 139$ ($r^2 = 0.9977$), respectively. The retention time of CPT-carboxylate and CPT-lactone was 2.1 ± 0.1 min and 7.3 ± 0.1 min.

3. Results and discussion

3.1. Effect of PAMAM concentration on solubility of CPT

The effect of PAMAM dendrimer on solubility of CPT was carried out using G4 PAMAM dendrimer of theoretical molecular weight 14,215 Da and 64 amine groups in the outer shell (Table 1), and the results are shown in Fig. 1. The solubility of CPT (both CPT-carboxylate and CPT-lactone) increased significantly in the presence of G4 PAMAM dendrimer (The solubility of CPT in the absence of dendrimers

Table 1
The characteristic data of G4, G5 and G6 PAMAM dendrimers in the present study

Generation	Molecular formula	Molecular weight	Number of terminal amino/ester groups	Number of total amino groups	Radius (Å)
G4	$C_{622}H_{1248}O_{124}N_{250}$	14,215	64	124	22.5
G5	$C_{1262}H_{2528}O_{252}N_{506}$	28,826	128	252	27
G6	$C_{2542}H_{5088}O_{508}N_{1018}$	58,048	256	508	33.5

was 2.94 ± 0.18 μg/ml for CPT-lactone and 6.39 ± 2.11 μg/ml for CPT-carboxylate in this study, these data are not shown in Figs. 1–3.). It was reported that the carboxylate-terminated G4 polyester dendrimer could enhance the solubility of CPT by 10-fold at a concentration of 1.4×10^{-3} M [41]. However, G4 PAMAM dendrimer increased the solubility of CPT by over 20-fold even at a low concentration of 7×10^{-5} M in this study. This result was presumably due to the existence of several interaction mechanisms between PAMAM dendrimers and CPT molecules. First, PAMAM dendrimers have large numbers of primary amines on their surface, which could interact electrostatically with the carboxyl group in the CPT-carboxylate molecules [15,26]; Second, PAMAM dendrimers possess empty internal cavities and an open structure [27], due to these specific and interesting properties of PAMAM dendrimers, the hydrophobic cavities in PAMAM dendrimers can keep small guest molecules such as CPT-lactone inside and make dendrimers suitable for enhancing the solubility of hydrophobic drug molecules in aqueous medium [28,29]; Third, there are tertiary amines in these internal cavities, which could interact with the atoms of the CPT-lactone molecules by hydrogen bond formation [28]. Therefore, PAMAM dendrimers possess open and internal cavities and many functional terminal groups, which are responsible for high solubility and reactivity. These specific properties make the PAMAM series interesting candidates as solubility enhancers of CPT.

3.2. Effect of PAMAM generation on solubility of CPT

The effect of various generations of PAMAM dendrimers (G4–G6) on the process was investigated. The results are shown in Figs. 1–3, from which it is clear that the solubility of CPT was affected by the generation of PAMAM dendrimer. The solubility of CPT in higher generation PAMAM solution was in fact higher that those in lower ones. The solubility of hydrophobic compounds in dendrimer solutions has previously been shown to depend on the dendrimer generation (size) [23,28]. Since the number of primary and tertiary amines in the dendrimer increases with generation size (Table 1), at a given pH condition, higher generation dendrimer has a tendency to entrap more hydrophobic compound inside than lower generations. Also, the solubility of CPT 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 to absorb and interact with the CPT molecule. In this way, we could explain why higher generation dendrimers could enhance the solubility of CPT more efficiently than lower generations.

4. Conclusion

Although dendrimer drug delivery is in its infancy, it offers several attractive features [30,31]. It provides a uniform

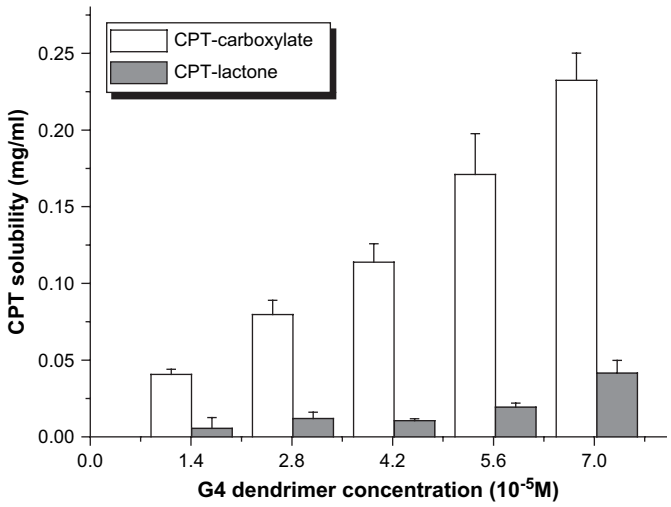

Fig. 1. Solubility of CPT (CPT-lactone and CPT-carboxylate) in the presence of G4 PAMAM dendrimers.

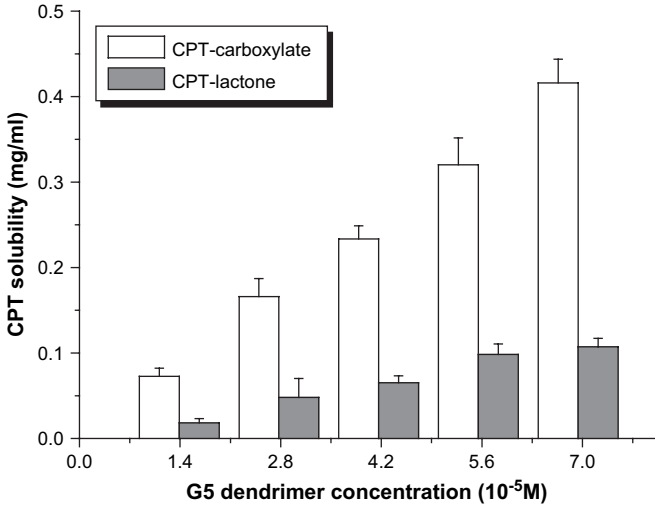

Fig. 2. Solubility of CPT (CPT-lactone and CPT-carboxylate) in the presence of G5 PAMAM dendrimers.

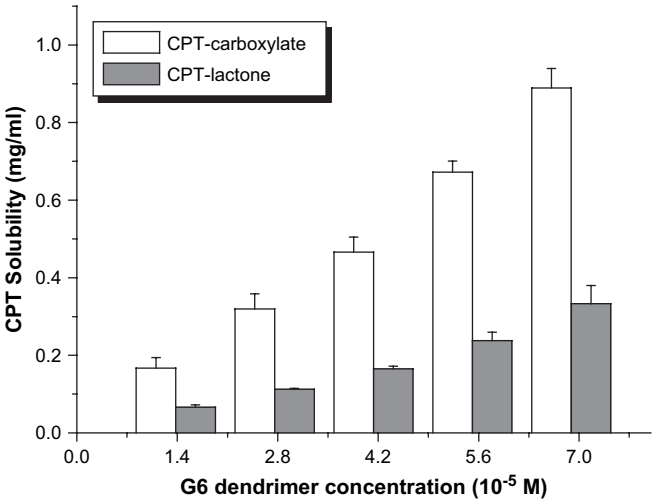

Fig. 3. Solubility of CPT (CPT-lactone and CPT-carboxylate) in the presence of G6 PAMAM dendrimers.

platform for drug attachment that has the ability to bind and release drugs through several mechanisms. Our work demonstrated that encapsulation/complexation of CPT molecules into/with dendrimers led to excellent solubility of these drugs. We are in the process of conducting *in vitro* cell studies and pre-clinical testing to evaluate the potential of dendrimers as carriers for CPT. Although toxicity problems may exist, modification of the structure of dendrimers should resolve this issue [38–40].

Acknowledgements

This work was financially supported in part by Natural Science Foundation of Anhui Province (No. 070413112), the Innovation Foundation from Hefei National Laboratory for Physical Sciences at Microscale (C07-06), and the Innovation Foundation of Graduate Student in University of Science and Technology of China (KD2004035). We thank Mr. Zhenhua Xu for his useful discussions and kind proof-reading of the manuscript.

References

[1] P. Opanasopit, T. Ngawhirunpat, A. Chaidedgumjorn, T. Rojanarata, A. Apirakaramwong, S. Phongying, C. Choochottiros, S. Chirachanchai, Incorporation of camptothecin into *n*-phthaloyl ChitoSAN-g-mPEG self-assembly micellar system, European Journal of Pharmaceutics and Biopharmaceutics 64 (2006) 269–276.

[2] M. Berrada, A. Serreqi, F. Dabbarh, A. Owusu, A. Gupta, S. Lehnert, A novel non-toxic camptothecin formulation for cancer chemotherapy, Biomaterials 26 (2005) 2115–2120.

[3] A.P. Zhu, J.S. Liu, W.H. Ye, Effective loading and controlled release of camptothecin by *O*-carboxymethylchitosan aggregates, Carbohydrate Polymers 63 (2006) 89–96.

[4] K. Kumi, W. Masato, Y. Tatsuhiro, Y. Masayuki, O. Praneet, O. Teruo, M. Yoshie, Enhanced antitumor effect of camptothecin loaded in long-circulating polymeric micelles, Journal of Controlled Release 112 (2006) 329–332.

[5] M. Watanabe, K. Kawano, M. Yokoyama, P. Opanasopit, T. Okano, Y. Maitani, Preparation of camptothecin-loaded polymeric micelles and evaluation of their incorporation and circulation stability, International Journal of Pharmaceutics 308 (2006) 183–189.

[6] D.A. Tomalia, H. Baker, J. Dewald, A new class of polymers: starburst-dendritic macromolecules, Polymer Journal 17 (1985) 117.

[7] D.A. Tomalia, H. Baker, J.R. Dewald, Dendritic molecules: synthesis of starburst dendrimer, Macromolecules 19 (1986) 2466.

[8] D.A. Tomalia, J.R. Dewald, Dense Star Polymers Having Two Dimensional Molecular Diameter, U.S. Patent 4,587,329 (1986).

[9] 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, Angewandte Chemie International Edition in English 29 (1990) 138.

[10] P. Furuta, J.M.J. Fréchet, Controlling solubility and modulating peripheral function in dendrimer encapsulated dyes, Journal of the American Chemical Society 125 (2003) 13173–13181.

[11] S. Svenson, D.A. Tomalia, Dendrimers in biomedical applications-reflections on the field, Advanced Drug Delivery Reviews 57 (2005) 2106–2129.

[12] E. Roseita, D.A. Tomalia, Poly (amidoamine) (PAMAM) dendrimers: from biomimicry to drug delivery and biomedical applications, Drug Delivery Today 6 (2001) 427–436.

[13] K.P. Anil, I.J. Majoros, J.R. Baker, Dendritic polymer macromolecular carriers for drug delivery, Current Opinion in Chemical Biology 6 (2002) 466–471.

[14] R.G. Elizabeth, J.M.J. Fréchet, Dendrimers and dendritic polymers in drug delivery, Drug Delivery Today 10 (2005) 35–43.

[15] O.M. Milhem, C. Myles, N.B. McKeown, Polyamidoamine starburst dendrimers as solubility enhancers, International Journal of Pharmaceutics 197 (2000) 239–241.

[16] P. Kolhe, E. Misra, R.M. Kannan, S. Kannan, M. Lieh-Lai, Drug complexation, in vitro release and cellular entry of dendrimers and hyperbranched polymers, International Journal of Pharmaceutics 259 (2003) 143–160.

[17] S. Kannan, P. Kolhe, V. Raykova, M. Glibatec, R.M. Kannan, M. Lieh-Lai, D. Bassett, Dynamics of cellular entry and drug delivery by dendritic polymers into human lung epithelial carcinoma cells, Journal of Biomaterials Science, Polymer Edition 15 (2004) 311–330.

[18] Y.Y. Cheng, T.W. Xu, Dendrimers as potential drug carriers. Part I. Solubilization of non-steroidal anti-inflammatory drugs in the presence of polyamidoamine dendrimers, European Journal of Medicinal Chemistry 40 (2005) 1188–1192.

[19] M.L. Ma, Y.Y. Cheng, Z.H. Xu, P. Xu, H.O. Qu, Y.J. Fang, T.W. Xu, L.P. Wen, Evaluation of polyamidoamine (PAMAM) dendrimers as drug carriers of anti-bacterial drugs using sulfamethoxazole (SMZ) as a model drug, European Journal of Medicinal Chemistry 42 (2007) 93–98.

[20] Y.Y. Cheng, H.O. Qu, M.L. Ma, Z.H. Xu, P. Xu, Y.J. Fang, T.W. Xu, L.P. Wen, Polyamidoamine (PAMAM) dendrimers as biocompatible carriers of quinolone antimicrobials: an in vitro study, European Journal of Medicinal Chemistry 42 (2007) 1032–1038.

[21] N. Malik, E.G. Evagorou, R. Duncan, Dendrimer–platinate: a novel approach to cancer chemotherapy, Anti-Cancer Drugs 10 (1999) 767–776.

[22] R.X. Zhuo, B. Du, Z.R. Lu, In vitro release of 5-fluorouracil with cyclic core dendritic polymer, Journal of Controlled Release 57 (1999) 249–257.

[23] C. Kojima, K. Kono, K. Maruyama, T. Takagishi, Synthesis of polyamidoamine dendrimers having poly (ethylene glycol) grafts and their ability to encapsulate anticancer drugs, Bioconjugation Chemistry 11 (2000) 910–917.

[24] Y.Y. Cheng, J.P. Yang, Solubilization of non-steroidal anti-inflammatory drugs in the presence of tween series surfactants, Physics and Chemistry of Liquids 44 (2006) 249–256.

[25] J.C. Kang, V. Kumar, D. Yang, P.R. Chowdhury, R.J. Hohl, Cyclodextrin complexation: influence on the solubility, stability and cytotoxicity of camptothecin, an antineoplastic agent, European Journal of Pharmaceutical Sciences 15 (2002) 163–170.

[26] Y.Y. Cheng, T.W. Xu, R.Q. Fu, Polyamidoamine dendrimers used as solubility enhancers of ketoprofen, European Journal of Medicinal Chemistry 40 (2005) 1390–1393.

[27] A.M. Naylor, I. Goddard, D.A. Tomalia, Starburst dendrimers 5: molecular shape control, Journal of the American Chemical Society 111 (1989) 2339–2341.

[28] D. Bharathi, A.H. Ronald, M.V. Melgardt, The effect of PAMAM dendrimer generation size and surface functional group on the aqueous solubility of nifedipine, International Journal of Pharmaceutics 284 (2004) 133–140.
[29] Y.Y. Cheng, T.W. Xu, Solubility of nicotinic acid in polyamidoamine dendrimer solutions, European Journal of Medicinal Chemistry 40 (2005) 1384–1389.
[30] Y.Y. Cheng, N. Man, T.W. Xu, R.Q. Fu, X.Y. Wang, X.M. Wang, L.P. Wen, Transdermal delivery of non-steroidal anti-inflammatory drugs mediated by polyamidoamine (PAMAM) dendrimers, Journal of Pharmaceutical Sciences 96 (2007) 565–602.
[31] N. Man, Y.Y. Cheng, T.W. Xu, Y. Ding, Z.W. Li, G.Y. Huang, Y.Y. Shi, L.P. Wen, Dendrimers as potential drug carriers. Part II. Prolonged delivery of Ketoprofen by in vitro and in vivo studies, European Journal of Medicinal Chemistry 41 (2006) 670–674.
[32] W. Tong, L. Wang, M.J. D'Souza, Evaluation of PLGA microspheres as delivery system for antitumor agent-camptothecin, Drug Development and Industrial Pharmacy 29 (2003) 745–756.
[33] A.M. Sætern, N.B. Nguyen, A. Bauer-Brandl, M. Brandl, Effect of hydroxypropyl-β-cyclodextrin-complexation and pH on solubility of camptothecin, International Journal of Pharmaceutics 284 (2004) 61–68.
[34] R. Cortesi, E. Esposito, A. Maietti, E. Menegatti, C. Nastruzzi, Formulation study for the antitumor drug camptothecin: liposomes, micellar solution, and a microemulsion, International Journal of Pharmaceutics 159 (1997) 95–103.
[35] R. Barreiro-Iglesias, L. Bromberg, M. Temchenko, T.A. Hatton, A. Concheiro, C. Alvarez-Lorenzo, Solubilization and stabilization of camptothecin in micellar solutions of pluronic-g-poly(acrylic acid) copolymers, Journal of Controlled Release 97 (2004) 537–549.
[36] Q.Y. Li, Y.G. Zu, R.Z. Shi, L.P. Yao, Review camptothecin: current perspectives, Current Medicinal Chemistry 13 (2006) 2021–2039.
[37] A. Hatefi, B. Amsden, Camptothecin delivery methods, Pharmaceutical Research 19 (2002) 1389–1399.
[38] H.T. Chen, M.F. Neerman, A.R. Parrish, E.E. Simanek, Cytotoxicity, hemolysis, and acute in vivo toxicity of dendrimers based on melamine, candidate vehicles for drug delivery, Journal of the American Chemical Society 126 (2004) 10044–10048.
[39] O.L. Padilla de Jesus, H. Ihre, L. Gagne, J.M.J. Frechet, F.C.J. Szoka, Polyester dendritic systems for drug delivery applications: in vitro and in vivo evaluation, Bioconjugate Chemistry 13 (2002) 453–461.
[40] R. Duncan, L. Izzo, Dendrimer biocompatibility and toxicity, Advanced Drug Delivery Reviews 57 (2005) 2215–2237.
[41] M.T. Morgan, Y. Nakanishi, D.J. Kroll, A.P. Griset, M.A. Carnahan, M. Wathier, N.H. Oberlies, G. Manikumar, M.C. Wani, M.W. Grinstaff, Dendrimer-encapsulated camptothecins: increased solubility, cellular uptake, and cellular retention affords enhanced anticancer activity in vitro, Cancer Research 66 (2006) 11913–11921.