J Incl Phenom Macrocycl Chem (2014) 79:65–73
DOI 10.1007/s10847-013-0324-z

ORIGINAL ARTICLE

Evaluation of different PAMAM dendrimers as molecular vehicle of 1,2,4-triazine *N*-oxide derivative with potential antitumor activity

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Received: 15 February 2013 / Accepted: 15 April 2013 / Published online: 26 April 2013

Abstract The interaction between a 1,2,4-triazine *N*-oxide derivative, that holds potential antitumor activity under hypoxic conditions, and diverse polyamidoamine (PAMAM) dendrimers were investigated with the purpose of select the most appropriate macromolecule to act as potential molecular carrier of this active compound. The results shows that dendrimers with amine terminal groups (PAMAM-AT G = 3) and dendrimers with carboxylate terminal groups (PAMAM-CT G2.5 and G4.5) produces triazine derivative hydrolysis, even in buffered medium, and are not suitable as carriers. In contrast, dendrimers with neutral end groups (PAMAM-OHT) shows stable association with the active compound, making this dendrimer a possible medium for triazine carriage.

Keywords Dendrimers · PAMAM · Triazine *N*-oxide · Host–guest association · Antitumor drug stability

Introduction

Triazines derivatives possess an essential position in modern medicinal chemistry, because of their high potential for biological activity [1]. In particular, 1,2,4-triazines, condensed with one or more heterocycles, found application in many fields as pharmaceutical, herbicides, pesticides and dyes [2]. For example, a series of novel tricyclic [1, 2, 4]triazine 1,4-dioxides were evaluated and showed hypoxia-selective cytotoxicity in vitro [3], while pyrrolo[2,1-*f*][1, 2, 4]triazines C-nucleoside have shown a pronounced in vitro growth inhibitory activity against leukaemia cell lines [4]. As well, 3-alkyl 1,2,4-benzotriazine 1,4-dioxides are selectively toxic under hypoxia with good extravascular transport [5]. For instance, 3-amino-1,2,4-benzotriazine 1,4-di-*N*-oxide, also known as Tirapazamine, shows high selective toxicity toward cells in hypoxic conditions, both in vitro and in tumors in vivo, and is currently undergoing phase II and III clinical trials as an antitumor agent [6]. Thus, it is noteworthy that many potential drugs have been modelled on 1,2,4-triazines, particularly in cancer and viral research [2]. Nevertheless, these potential therapeutic agents, like most of chemotherapeutic drugs, often also kill healthy cells and cause toxicity to the patient. It would therefore be desirable to develop chemotherapeutics formulations that can passively target cancerous cells as nanocarriers. Passive targeting exploits the characteristic features of tumor biology that allow nanocarriers to accumulate in the tumor by the enhanced permeability and retention (EPR) effect [7]. Diverse therapeutic nanocarriers based on this strategy were evaluated and methods of further enhancing targeting of drugs to cancer cells were intensely investigated [8, 9]. Dendrimers are a class of macromolecules extensively studied for this purpose [10]. They represent the so-called 4th architectural class of polymers and the newest category of drug delivery carriers. Dendrimers have a well-defined highly branched structure suitable for pharmaceutical applications. They are small in size; with low polydispersity which is a crucial factor to the reproducibility of the

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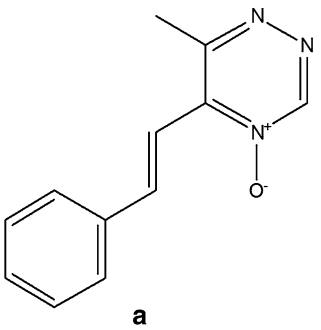

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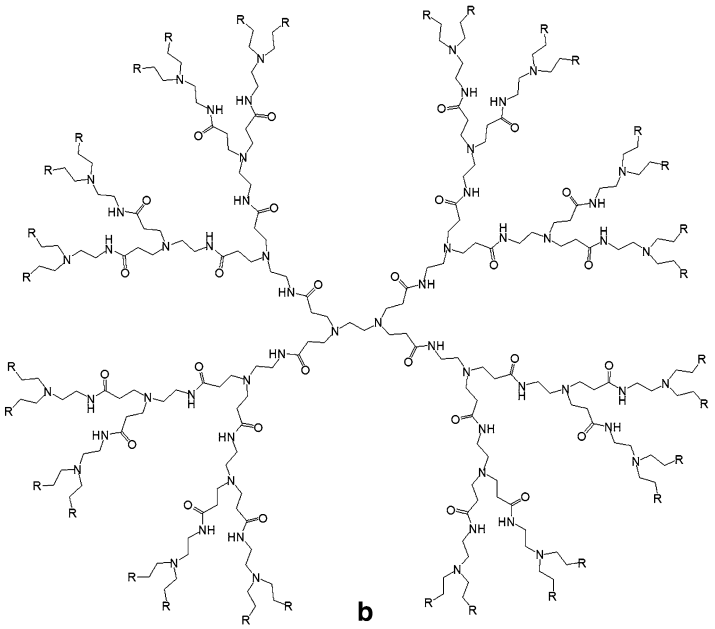

b

Fig. 1 Chemical structure of benzo[1, 2-e]1, 2, 4-triazine N(1)-oxide (triazine) **(a)** and chemical structure of studied dendrimers PAMAM-AT, PAMAM-CT and PAMAM-OHT **(b)**

R = $CONHCH_2CH_2NH_2$	PAMAM -AT (G = 3)
R = $CONHCH_2CH_2OH$	PAMAM - OHT(G = 3)
R = COONa	PAMAM - CT (G = 2.5)
R = $CONHCH_2CH_2N(CH_2CH_2CONHCH_2CH_2N(CH_2CH_2COONa)_2)_2$	PAMAM -CT (G = 4.5)

pharmacokinetic behaviour of the encapsulated drug [11]. The use of dendrimers as drug carriers allows the delivery of a high payload of drug with reduced cytotoxic side effect [10, 12].

The family of dendrimers most investigated in drug delivery is the poly(amido amine) dendrimers (PAMAM). PAMAM dendrimers are biocompatible, non-immunogenic, water soluble and possess terminal modifiable amine functional groups for binding various targeting or guest molecules [13, 14]. The high density of amino groups and internal cavities in PAMAM dendrimers is expected to have potential applications in enhancing the aqueous solubility of lipophilic compounds [8, 15]. For example, PAMAM dendrimers have been used to encapsulate and solubilize diverse drugs such as nifedipine [16], ibuprofen[17], metotrexate [18] and indomethacin [19].

In this work we investigate the interaction between diverse PAMAM dendrimers and 1,2,4-triazine *N*-oxide derivative (Fig. 1a), synthesized in order to obtain compounds as selective hypoxic cell cytotoxicity. This drug present antitumor activity, but showed low cytotoxic selectivity against V79 cells in hypoxic conditions compared with Tirapazamine, the reference bioreductive drug, probably due to the low water solubility and instability [20, 21]. Therefore, to encapsulate this type of compound in water–soluble carrier could improve their activity. The objective of this study is to evaluate the ability of PAMAM dendrimers with amine and carboxyl terminal groups and PAMAM derivatives modified by hydroxyl (Fig. 1b) to associate with triazine derivative with the purpose of select the most appropriate macromolecule to act as potential molecular carrier.

Materials and methods

General

PAMAM-AT (G = 3), PAMAM-OHT (G = 3), PAMAM-CT (G = 2.5) and PAMAM-CT (G = 4.5) dendrimers in methanol solution were obtained from Sigma-Aldrich. The active derivative (E)-2-phenylethenyl-1,2,4-triazine *N*1 oxide (triazine) (Fig. 1a) was prepared as previously described [20] and stored at room temperature under vacuum.

The organic solvents chloroform, dichloroethane, ethyl acetate, ethyl ether, ethanol, isopropanol, tetrahydrofuran, butanol, acetonitrile, methanol, dimethylformamide, toluene, benzene, dimethyl sulfoxide, butylamine and tributylamine were purchased from Sintorgan (HPLC quality) and were used without further purification. The UV cut-off point of the solvents in a UV cell of 10 mm against air was used as purity criteria. Deionized water was obtained from Elga Classic equipment.

UV visible spectroscopic measurements were performed using a Shimadzu UV 2401 PC spectrophotometer at 20.0 ± 0.2 °C. Absorption maximum frequencies (υ_{max}) were measured by taking the middle point between the two positions of the band where the absorbance is equal to 0.90 A_{max} [22].

The charge density distributions were predicted from theoretical calculations performed by semi-empirical molecular orbital methods AM1 [23]. Calculations were performed starting from Standard bond lengths and bond angles. All geometries were fully optimized by minimizing the energy with respect to the geometrical variables without symmetry constraint, using a gradient of 0.01 kcal/mol and the Polak–Ribierie algorithm as convergence criteria. Semiempirical calculations were carried out using the HyperChem software, running in a personal computer.

Sample preparation methods

Stock solution of triazine was prepared dissolving the guest in methanol at 2.0×10^{-3} M, and stored in darkness. Appropriate aliquots of this solution were transferred into volumetric flasks and methanol evaporated off under nitrogen stream. The samples were diluted to volume with corresponding solvent, sonicated for 10 min, and stored to room temperature in darkness until further use. The electronic absorption spectrum of triazine was recorded in solvents with different polarity listed in Table 1.

Saturated solutions of triazine in water were prepared adding a solute excess in pure water, leaving to ultrasound bath for 20 min, and then allowing equilibration overnight in darkness. Small amount of drug precipitated from solution was removed via filtration through a 0.45 μm Millipore membrane. Concentration was determinate by spectrophotometry at $\lambda_{max} = 364$ nm.

To prepare the triazine/dendrimer/water samples, appropriate volumes of the solute stock solutions in methanol were transferred into 5 mL volumetric flasks, and the solvent evaporated off under nitrogen stream. The samples were diluted to the appropriate volume with aqueous stock solution of PAMAM dendrimer, sonicated for 10 min and vigorously stirred for 12 h, then allowed to equilibrate in darkness overnight. Small amount of drug precipitated from solution and was removed via filtration through a 0.45 μm Millipore membrane.

Table 1 Solvent parameters α, β and π* and wavenumber of the absorption maxima corresponding to the band 2 of triazine spectrum in all used solvents

Solvent	Wavenumber (cm^{-1})	Solvent parameters[1]		
		α	π*	β
Methanol	28409.0	0.93	0.60	0.62
Ethyl acetate	28761.4	0.00	0.55	0.45
Dimethylformamide	28531.0	0.00	0.88	0.69
Butanol	28089.9	0.79	0.47	0.88
Benzene	28733.7	0.00	0.59	0.10
Isopropanol	28169.0	0.76	0.48	0.95
Acetonitrile	28902.0	0.19	0.75	0.31
Toluene	28768.3	0.00	0.54	0.11
Tetrahydrofuran	28727.8	0.00	0.58	0.55
Dimethyl sulfoxide	28328.6	0.00	1.00	0.76
Chloroform	28089.9	0.44	0.58	0.00
Ethanol	28328.6	0.83	0.54	0.77
Dichloroethane	28581.5	0.00	0.81	0.00
Water	27473.0	1.17	1.09	0.18
Tributylamine	28985.5	0.00	0.16	0.62

[1] α = solvent hydrogen-bond donor parameter, π* = solvent polarity polarizability parameter and β = solvent hydrogen-bond acceptor parameter, from ref [24]

In vitro release studies

The release rate of triazine from PAMAM dendrimer in deionized water solution was measured by the following procedures. One milliliter of dendrimer/triazine aqueous solutions or triazine in deionized water were transferred into a dialysis bag with a molecular weight cut off (MWCO) of 1,000 Da, followed by immersion of the dialysis bag into a container filled with 10 mL deionized water. This system was continually stirred using a small magnetic stir bar to prevent the formation of an unstirred water layer at the membrane/outer solution interface. The diffusion to the outer solution was determinate withdrawing an aliquot of 3 mL from the outer phase of the dialysis

bag at specific time intervals and the medium was replaced by deionized water. The triazine concentrations in these solutions were analyzed by spectroscopic method.

Results and discussion

Study of triazine-medium interactions

In order to analyze and characterize the triazine-medium interactions we develop spectroscopic studies of the triazine derivative using Kamlet and Taft's solvatochromic comparison method [24]. By this method empirical parameters are used to quantify specific interactions and separate them from polarity–polarizability effects. The identification and analysis of the potential drug-dendrimer interactions nature allows to understand characteristics of the conjugate, and to design strategies for encapsulation and controlled release of active compounds [25–27].

Electronic absorption spectra of triazine were recorded in solvents of different polarity and hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA) ability. The absorption spectrum of triazine consists of two characteristic UV bands, an intense band ($\lambda_{max1} = 267$ nm, water) and a weaker band ($\lambda_{max2} = 364$ nm, water).

It was found that the longer wavelength absorption maximum, band 2, is strongly dependent on solvent polarity. Some representative spectra in different solvents are show in Fig. 2. Triazine absorption exhibits a bathochromic shift with the increase of medium polarity, consistent with a $\pi \rightarrow \pi^*$ transition [28], similar to those observed for tirapazamine compounds [29].

Kamlet and Taft's linear free energy relationship method rationalises solvent effects in terms of a linear combination, which depends on three fundamental indexes: the π^* scale (solvent dipolarity–polarizability parameter), which measures the ability of the medium to stabilise the charge on a dipole by virtue of its dielectric effects; the α scale (solvent hydrogen-bond donor, HBD, acidity), which describes the solvent's ability to donate a proton in a hydrogen bond; and the β scale (solvent hydrogen-bond acceptor, HBA, basicity) provides a measure of the solvent's ability to accept a proton (donate an electron pair) in a hydrogen bond [24]. These solvatochromic parameters are used in linear solvation energy relationships of the general form:

$$XYZ = XYZ_0 + s\pi^* + a\alpha + b\beta \quad (1)$$

where XYZ is the property to be correlated; s, a and b coefficients measure the relative susceptibilities of XYZ to the indicated solvent property scales. The π^*, α, β parameters of the used solvents are summarized in Table 1, together with the wavenumber (cm^{-1}) of the absorption maxima corresponding to the band 2 of triazine spectrum.

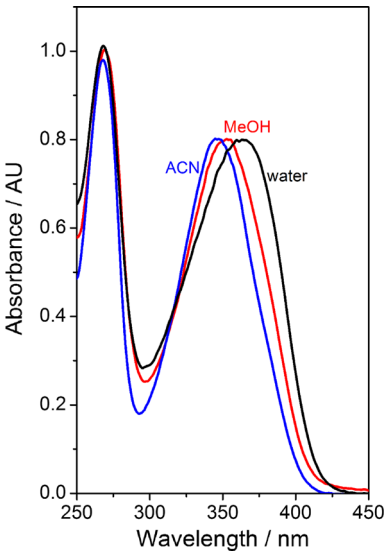

Fig. 2 Electronic absorption spectra of triazine in water, acetonitrile (ACN) and methanol (MeOH)

The results of the stepwise regression applied to Eq. (1), with the values of absorption maxima frequencies (υ_{max}) for triazine in different pure solvents, are given by Eq (2)

$$\upsilon_{max} = (29,163 \pm 110) - (647 \pm 86)\,\alpha - (764 \pm 160)\,\pi^* \quad (2)$$

where the number of solvents included in the correlation was 13, and the correlation coefficient obtained was $r = 0.8986$. The υ_{max} for triazine shows correlation only with π^* and α solvent parameters. No statistical significance was observed for β parameter, since that the b value is smaller than the error, according to the test [24].

The obtained results indicate that the triazine derivative is sensitive to the environment polarity, and that is able for to establish hydrogen bond interactions, acting as acceptor. This result is consistent with the fact that molecules possessing *N*-oxide function are highly polar, and readily form hydrogen bond with hydrogen-bond donor species.

Hydrogen bonding acceptor nature of triazine also can be predicted from the analysis of local charge density, (Fig. 3), calculated by semi empirical molecular orbital methods AM1 [23]. This semi-empirical method does not describe the solvation processes, but it may provide information about the interaction sites, the bond distances, and charge distribution of the different atoms present in the main structure. In order to suggest a possible localization of the hydrogen-bond interactions, the calculated charge density distributions for different atoms of the active molecule were analyzed, and a negative charge excess is localized on the oxygen of the *N*-oxide group.

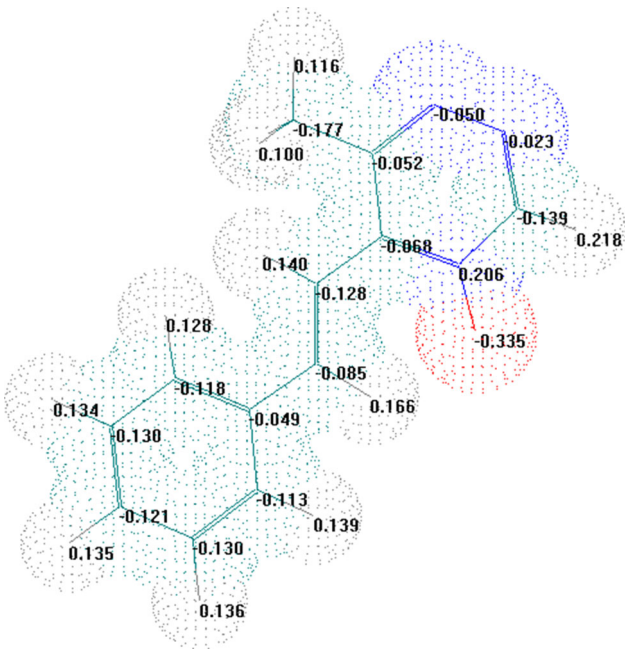

Fig. 3 Charge distribution of 1, 2, 4-triazine *N*-oxide derivative calculated using semiempirical AM1 method

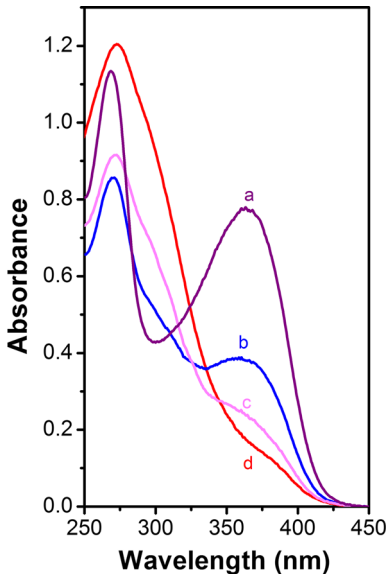

Fig. 4 Electronic absorption spectra of triazine in 1×10^{-4} M aqueous solutions of PAMAM-AT (G = 3) took at different times. Immediately after solution preparation **(a)**, and after three **(b)**, five **(c)** and ten **(d)** days

Therefore, based on the solvatochromic studies and semiempirical calculations, it is possible to predict that the antitumor compound will interact with the dendrimeric nanocarriers not only by the lipophilic–hydrophilic balance, but also through specific hydrogen bond interaction between the *N*-oxide group in active compound and the amine and/or amide residues in the PAMAM dendrimers. The existence of these specific interactions can promote drug-dendrimer association leading enhance of active compound solubility.

Triazine-dendrimers association

The association between dendrimers and triazine was studied by spectroscopic methods for the different triazine-dendrimer systems in aqueous solution, using full PAMAM G = 3 and half generation PAMAM G = 2.5 and 4.5 dendrimers (Fig. 1b). The studied dendrimers consists of tertiary amines and amide groups in their interior, and present amine and carboxylate groups in their periphery. As was already mentioned, the internal and peripheral groups can establish specific interaction with *N*-oxide hydrogen bonds acceptor group present in triazine.

Absorption spectra of triazine in aqueous solutions of PAMAM-AT (G = 3), prepared as described in materials and methods section, acquired at different times after dissolution, are shown in Fig. 4. We found that the absorption spectrum of the triazine undergoes changes with the time: the band with maximum at 364 nm decreases, meanwhile the development of a shoulder around 300 nm generates broadening of the band centered at 269 nm. This fact indicates that the triazine derivative is not stable in aqueous solutions of PAMAM-AT (G = 3) dendrimer, and that an irreversible degradation of the active compound occurs.

In contrast, control experiments about triazine stability in deionized water showed that its electronic spectra did not exhibited any change along several weeks. Similar spectral changes due to solute degradation were observed when the association of triazine with PAMAM-CT (G = 2.5 and 4.5) dendrimers was studied in aqueous media (Fig. 5). Chupakhin et al. [30] reported that 1,2,4-triazine *N*-oxides undergo hydrolysis in both acid and basic media. In our case the pH of the dendrimers aqueous solutions were 9.13, 9.80 and 10.02 for PAMAM-AT (G = 3), PAMAM-CT (G = 2.5) and PAMAM-CT (G = 4.5) respectively, which are in agreement with those already reported [31]. Therefore, it could be possibly that the dendrimers are involved in a hydrolysis process of triazine derivative, with the concomitant formation of *N*'-[3-(hydroxyimino)-5-phenylpent-4-en-2-ylidene]formylhydrazide [32] and the observed spectral changes are an evolution of triazine bands to the hydrolyzed triazine compound.

In order to analyze the triazine hydrolysis process, we followed the evolution of the absorption spectra of an aqueous triazine solution after the addition of KOH enough to reach a pH value of 10. Clear changes in the electronic spectra are observed in Fig. 6, indicating the transformation (basic hydrolysis) suffered by the compound. The final spectrum was quite similar to those obtained in the already described dendrimer media, which support our assumption

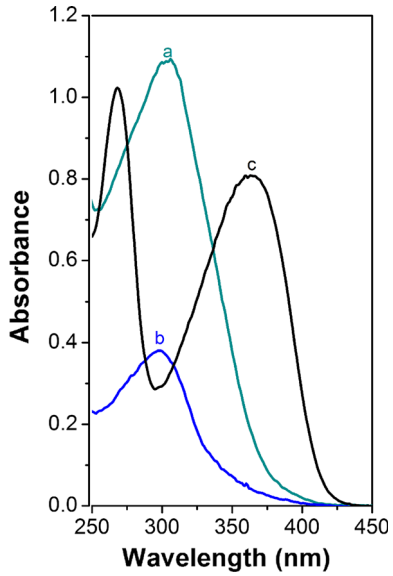

Fig. 5 Electronic absorption spectra of triazine in 1×10^{-4} M aqueous solutions of PAMAM-CT G = 4.5 **(a)** and G = 2.5 **(b)** took after 10 days of sample preparation. Spectrum of control solution in deionized water is introduced for comparison **(c)**

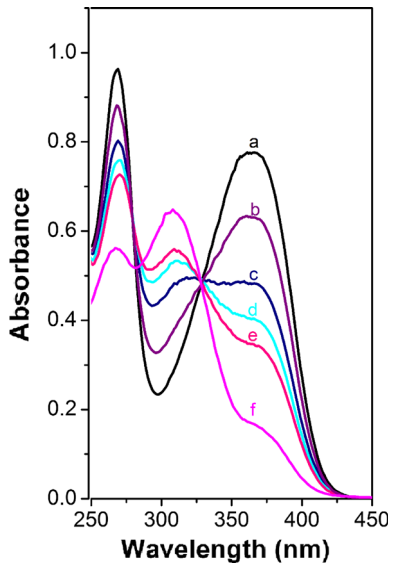

Fig. 6 Time-dependent change in electronic absorption spectra of triazine in aqueous solutions of KOH (pH = 10) measured at 0 **(a)**, 20 **(b)**, 60 **(c)**, 180 **(d)**, 420 min **(e)** and one day **(f)** after solution preparation

that in the presence of the studied PAMAM macromolecules triazine undergoes irreversible hydrolysis. Moreover, studies of triazine stability performed in aqueous solution of butylamine at a concentration equivalent to the number of terminal groups present in a solution of 1×10^{-4} M PAMAM-AT (G = 3) dendrimer, displayed a similar degradation.

Therefore, we attempted to study the drug-dendrimer association in buffer medium (Phosphate buffered saline, PBS, pH = 7.4). However, despite pH regulation, long term spectroscopic analysis also showed triazine degradation. As example Fig. 7 display the absorption spectrum of triazine in PBS buffered solution in the presence of PAMAM-AT (G = 3) dendrimer obtained after one week of solutions preparation. Similar degradation were observed for PAMAM-CT (G = 2.5 and 4.5) in PBS buffered solution. This fact indicates that the charged peripheral sites existent in PAMAM-AT and PAMAM-CT dendrimers could be responsible of antitumor derivative degradation. The high local concentration of multiple end-groups could promote the occurrence of multiple complexation events than occur simultaneously at these sites, which can lead to special types of multivalent interactions [33]. Klajnerta et al. demonstrated that both, carboxylate and amino end groups are ionized in water solutions and in buffer at pH 7.4, and that only hydroxy-terminated dendrimers bear no charge on their surface [34]. Besides all the analyzed dendrimers possess groups that could promote base-catalyzed hydrolysis, in PAMAM dendrimers the basicity of the inner amine residues are strongly dependent on the generation at which the tertiary amine belongs, and on the nature of the dendrimers′ terminal groups [35]. In the case of PAMAM-AT dendrimer, it possesses primary amine terminal groups with pKa around 8.5 [35, 36] which are able to promote triazine basic hydrolysis process. On the other hand, in full generation PAMAM dendrimers, the internal tertiary amines has a pKa $\approx$ 5.0 [35, 36], thus it is plausible to suppose that under our experimental conditions in PAMAM-OHT (G = 3) the internal amine groups remain neutral in aqueous solution. In PAMAM-CT dendrimer, besides the low basicity of the carboxylates groups [35], the tertiary amines adjacent to the terminal groups posses an enlarged basic capacity (pKa $\approx$ 8.0) [35] in comparison with full generation dendrimers, making possible basic hydrolysis of the active compound. Thus, our results remark the fact that the microheterogeneous environment in dendrimeric solutions can produce undesirable effects when these structures are used as carriers in drug delivery systems. In the present case, the interaction between the triazine derivatives and amino and carboxylated terminated dendrimers causes the degradation of active compound, and are not suitable to act as macromolecular vehicles for the antitumor compound.

In order to found a compatible drug carrier, we evaluated the interaction between the triazine derivative and neutral hydroxy-terminated dendrimer PAMAM-OHT (G = 3) (Fig. 1b), with similar core and branches, but with non ionized end groups. Fig. 8 shows the electronic absorption spectra of triazine in aqueous solution of PAMAM-OHT (G = 3) dendrimer (1×10^{-4} M) and in a

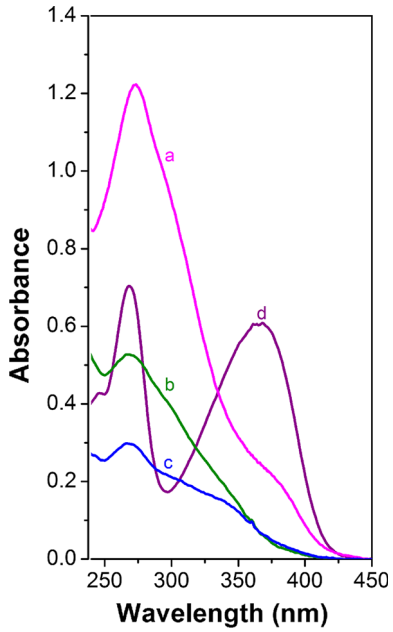

Fig. 7 Electronic absorption spectra of triazine in 1×10^{-4} M PAMAM-AT **(a)**, PAMAM-CT G = 4.5 **(b)** and PAMAM-CT G = 2.5 **(c)** aqueous buffered (pH = 7.4) solutions took after ten days of sample preparation. Control spectrum in buffered water solution **(d)** is introduced for comparison

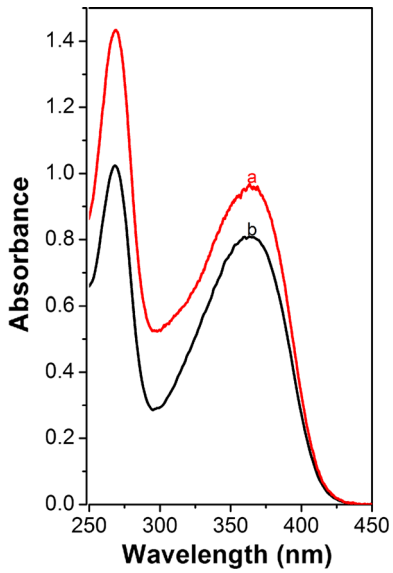

Fig. 8 Electronic absorption spectra of triazine in 1×10^{-4} M PAMAM-OHT **(a)** and in deionized water **(b)** solutions

saturated solution in pure water. The spectrum of triazine in the presence of the hydroxy-terminated dendrimer remains unchanged over time (several weeks), which indicates that there are not chemical degradation or structural changes in the active compound. Also, the increase in the absorbance can be attributed to triazine additional solubilization in the lipophilic interior of the dendrimer. Previous studies have shown that the internal microenvironment in PAMAM dendrimeric structure is less polar than the bulk aqueous phase [37]. On the other hand, the storage space in dendrimers is controlled by the geometrical parameters of the branch cell (branching angles, rotation angles and repeat-unit segment length) and by the shape and size of the available internal dendrimer microenvironment that influences the host–guest interactions. Often the maximum amount of entrapped guest molecules is proportional to the shape and size of the guest molecules. The molecular volume of triazine (V = 108.05 $Å^3$), calculated as described in materials and methods section, allows accessibility into the PAMAM-OHT (G = 3), thus the guest can be solubilized within the less polarity environment. Also, the incorporation of guests into the lipophilic interior of dendrimers can be reinforced by the triazine-dendrimer hydrogen bond, stabilizing the association of this host–guest system. PAMAM-OHT (G = 3) possess amide hydrogen bond donating groups into his cavities and hydroxyl in the periphery, which can form association with *N*-oxide residue in the active compound.

In order to quantify the effectiveness of PAMAM-OHT (G = 3) dendrimer in solubilize triazine, the enhancement solubilization factor (ESF) defined as the number of moles of compound solubilized per number of moles of dendrimer was calculated, using Eq. (3).

$$ESF = \frac{[H]_o - S_w}{[D]_w} \quad (3)$$

Where $[H]_o$ is the analytical concentration of the guest in aqueous solutions of dendrimers, $[D]_w$ is the concentration of dendrimers, and S_w is the water solubility of the guest. $S_w = 4 \times 10^{-4}$ M, was calculated as described in materials and methods section, $[H]_o$ was evaluated from the absorbance at $\lambda = 364$ nm (Fig. 8). An ES factor of one for triazine in PAMAM-OHT (G = 3) was found, which means that one active compound can be incorporated by each dendrimer nanocarrier. Comparable active compound loading capacity was reported by Morgan for Reichardt's day and for 10-hydroxycamptothecin in hydroxyl terminated poly(glycerol succinic acid) dendrimers [38]. Thus, the encapsulation behavior of PAMAM-OHT (G = 3) dendrimer towards triazines presents an operative strategy to prepare liquid formulation of triazine active compounds, and allow us to propose PAMAM-OHT (G = 3) dendrimer as appropriate macromolecular carrier for this family of active compounds.

In vitro release and stability tests of triazine-PAMAM-OHT (G = 3) complex

To evaluate the stability of triazine-dendrimer association, water solution samples were kept in capped vials at room

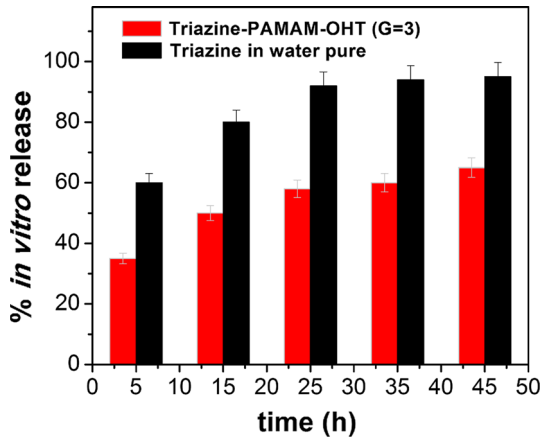

Fig. 9 In vitro release profile of triazine from 1×10^{-4} M PAMAM-OHT dendrimer solution compared with pure triazine in deionized water

temperature and periodically analyzed (once a day for 10 days, and after every week) up-to 10 weeks for any precipitation, turbidity, crystallization, spectral changes and drug leakage. No changes were noticed in the formulations. In separated experiments the systems were found to be sufficiently stable even at elevated temperatures up to 37 °C.

The in vitro drug release against deionized water was carried out as described in materials and methods section in order to evaluate the release characteristic of the dendrimer/triazine complexes, which is of critical importance in the design and optimization of drug delivery systems. Carriers with ideal sustained release characteristics can improve bioavailability and decrease side-effects of the administrated drug, and simplify the dosing schedules [18]. Figure 9 shows that nearly 98 % of free triazine molecules were released out of the dialysis bag within 45 h in the absence of dendrimer in the inner water solution. On the other hand, after the same time period, only 65 % of the drug was released out of the dialysis bag when PAMAM-OHT (G = 3) dendrimer is present in the solution. Moreover, under the same experimental conditions, the remaining 35 % is released a very low rate. Even after 24 h no appreciable changes are noticed in the amount of released triazine. The significant slow release of triazine from triazine/PAMAM-OHT solutions indicated the existence of a triazine-dendrimer association through hydrophobic/hydrogen-bond interactions. Binding sites in the carrier can prevent the rapid release of drug from the dendrimer/drug complex [18].

This study shows that the triazine molecules associated to the dendrimer structure remains unreleased of the formulation, and only free molecules are transferred outside the dialysis bag under the experimental conditions. Hence, the dendrimer-triazine interactions (polarity-polarizibility and hydrogen bond, as showed by solvathochromic analysis) are strong enough for to produce highly stable association in water solution. Thus, in the case of an eventual medical application of the drug-carrier formulation, the active compound would remain associated to the dendrimer. This is useful because the drug entrapped to the carrier can be retained in the tumor tissue for a longer time than the free drug with low-molecular weight that easily diffuses back out [39].

Conclusions

In the present study we investigate the association between a 1,2,4-triazine derivative that show potential hypoxic activity, and PAMAM dendrimers with amine, carboxylate and hydroxyl terminal groups. The specific and no specific intermolecular interactions between triazine and solvation medium were studied by the solvatochromic methods. The presence of PAMAM dendrimers with amine terminal groups (G = 3) and with carboxylate terminal groups (G = 2.5 and 4.5) in water solution induces irreversible degradation of the active compound, and these polymers are not suitable as carriers. On the other hand, dendrimers with neutral end groups (PAMAM-OHT) shows association with the active compound, without structural changes in the guest, and this formulation is stable over time. Thus, PAMAM-OHT has potential capacity to act as molecular vehicles of triazine derivatives that undergoes irreversible hydrolysis in the presence of charged dendrimers. This research provides new insights into dendrimer-drug delivery systems and will be helpful for design of new dendrimer/drug formulation for triazine derivatives.

Acknowledgments Financial support from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET-Argentina), Agencia Nacional de Promoción Científica y Tecnológica (FONCYT-Argentina), Secretaría de Ciencia y Técnica de la Universidad Nacional de Río Cuarto (SECYT-UNRC), is gratefully acknowledged.

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