Journal of Photochemistry & Photobiology, A: Chemistry 403 (2020) 112878

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, A: Chemistry

journal homepage: www.elsevier.com/locate/jphotochem

Synthesis, photophysical characterisation and antimicrobial activity of a new anionic PAMAM dendrimer

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ARTICLE INFO

Keywords:
1,8-Naphthalimide
Anionic PAMAM dendrimer
Antimicrobial activity
Antibacterial
Cotton fabric
Antimicrobial finishing

ABSTRACT

A new anionic dendrimer (D1) has been synthesized and characterized by modification of a poly(amidoamine) (PAMAM) dendrimer with 3-(6-nitro-1,3-dioxo-1H-benzo[de]isoquinolin-2(3 H)-yl)propanoic acid (NI1). Its photophysical characteristics have been investigated in organic solvents of different polarity and a positive solvatochromism has been observed. The effect of the pH medium on the fluorescence intensity has been investigated and it has been shown that in the highly alkaline medium the dendrimer emits intense fluorescence. The dendrimer and its monomeric structural analogue have been loaded on a cotton fabric and the release of substances from the surface of the cotton fabric has been investigated. The compounds have been screened for antimicrobial and cytotoxic activities. They have been found more active against Gram-positive bacteria. The dendrimer exhibits slightly lower activity than the monomeric analogue, but is found to be significantly less cytotoxic. The deposited on cotton fabric D1 prevents the formation of bacterial biofilm on the fabric surface.

1. Introduction

Dendrimers are a relatively new class of hyperbranched macromolecules with unique chemical structure and functional characteristics [1–3]. The increased attention to this class of compounds arises from the fact that they are a new form of organization of polymeric materials, the peculiarity of which is the combination of the properties of low molecular weight and high molecular weight substances. Compared to the small molecules and linear polymers with antimicrobial properties, dendrimers have the potential of a single molecule to carry a large dose of biologically active substance. In addition, dendrimer biocides are characterized not only by increased activity but by reduced toxicity, as well [4]. Dendrimers contain a large number of functional groups in the branches and in the periphery, which gives great opportunities for purposeful modification of their properties. The first one enables their binding to the functional groups of different matrix (for example textile materials). The second one allows multiple copies of a drug to unite in one molecule. This may induce a multivalent effect, reminiscent of the polyvalent interactions widely occurring in biological systems [2].

Functionalization of dendrimers with photoactive groups expand their application fields [5]. The chemical structure of the dendrimer molecules makes them relevant for different application in such fields as biomedicine, nanomedicine ecology, etc. [6–12]. Cationic or anionic dendrimer structures can be obtained by suitable chemical methods. They exhibit high antimicrobial activity against different pathogens [13–17]. Some of them have more pronounced activity to Gram-positive bacteria, while others are more active against Gram-negative bacteria.

The utilisation of antibacterial textiles in daily life, and especially in hospital and clinical practice, may prevent various diseases and bacterial infections. The production of antibacterial textiles is mainly achieved by treating textile materials with biologically active substances [18–21]. These substances can be attached to the surface of the textile materials through different interactions depending on their application.

In our laboratory we conduct systematic studies on the modification of dendrimers with 1,8-naphthalimide [22–25], acridine [26], benzanthrone [27,28], or 4-nitrobenzofurazan [29] with the aim of investigating the structure - properties relationship. These dendrimers have been probed as metal and/or pH detectors. In the last few years, our

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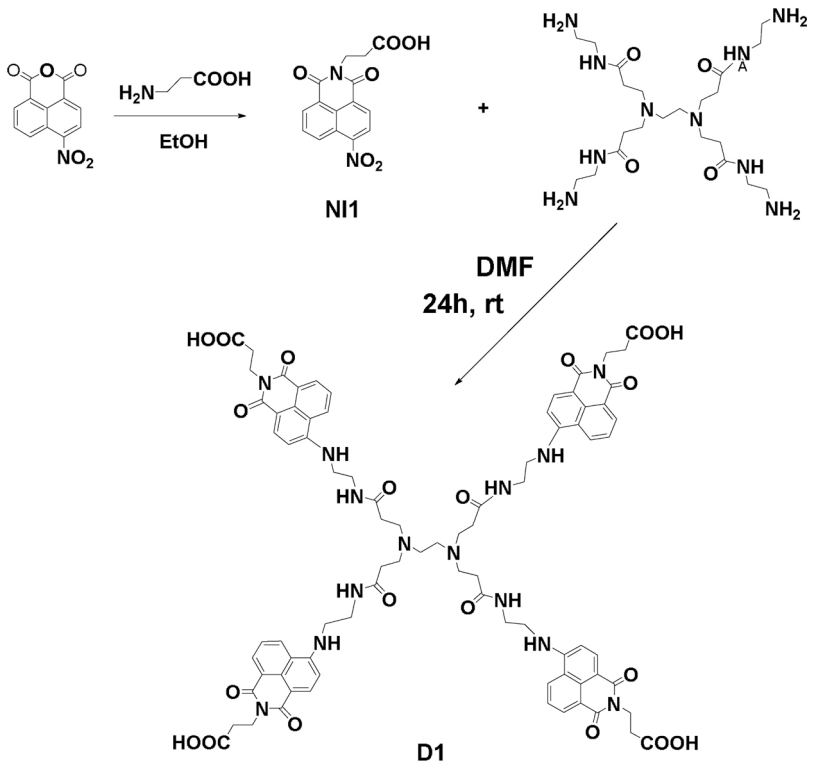
https://doi.org/10.1016/j.jphotochem.2020.112878
Received 22 July 2020; Received in revised form 21 August 2020; Accepted 27 August 2020
Available online 29 August 2020



Scheme 1. Synthesis of compounds NI1 and D1.

efforts have also been focused on the antimicrobial and anticancer activity of such systems [30–34]. This direction was dictated by the fact that pathogenic microorganisms are becoming more resistant to the antibiotics used in medical practice, which requires the search and study of new compounds with increased antimicrobial activity [35].

Derivatives of 1,8-naphthalimides are cyclic imides, characterized by hydrophobicity and large π-fused skeleton, which can easily interact with various biological systems through non-covalent interactions, such as π-π stacking. This determines the wide biological and biomedical interest to them [36–38].

This study aims to investigate a new anionic PAMAM dendrimer, peripherally modified with four 1,8-naphthalimide units. The basic photophysical characteristics of the dendrimer have been investigated in organic solvents of different polarity. The effect of pH has also been investigated. The antimicrobial activity of the dendrimer against different pathogens has been examined in a liquid medium and after deposition on a cotton fabric. By gaining insight into the factors that correlate with dendrimer structure one can design and further optimize their properties for various applications. A computer simulation technique (DFT calculations) has been used to predict some properties of the new PAMAM dendrimer (and to differentiate them from those of the 1,8-naphthalimide derivative, used for its peripheral functionalization) at the molecular level. DFT-based calculations provide valuable information about the configuration and conformation of the molecules and important insights into the electronic structure. To understand the relationship between electronic structure and photophysical behaviour, we have investigated theoretically (by means of popular and affordable DFT method) the 1,8-naphthalimide derivative and a model system, representing the studied new PAMAM dendrimer.

2. Experimental part

Detail description of used methods for spectral characterization microbiological activity and of compounds was done in Supplementary material.

2.1. Synthesis of 3-(6-nitro-1,3-dioxo-1H-benzo[de]isoquinolin-2(3 H)-yl)propanoic acid (NI1)

4-Nitro-1,8-naphthalic anhydride (2,43 g, 0.01 M), was dissolved at 50 ml ethanol and β-alanine (0.89 g, 0.01 M) was added. The mixture was refluxed 4 hours. After cooling, the formed needle crystals were filtered and washed with ethanol. Yield: 2.32 g, 74%.

FT-IR (KBr) cm^{-1}: 1741, 1708, 1654, 1590, 1524, 1346, 1300, 1231, 840, 784, 753.

^{1}H NMR (DMSO) δ (ppm): 2.66 (t. $J = 7.2$ Hz, 2H, HOOCCH2-), 4.24 (t. $J = 7.8$H, 2H, CH_2-N), 8.06 (dd, $J = 12.3$, 6.2 Hz 1H, Ar-H), 8.52 (d, $J = 8.2$ Hz, 1H, Ar-H), 8.57 (d, $J = 7.9$ Hz, 1H, Ar-H), 8.60(d, $J = 8.6$ Hz 1H, Ar-H), 8.67 (d, $J = 8.0$ Hz 1H, Ar-H),12.41 (s. 1H COOH);

^{13}C NMR (DMSO): δ (ppm) = 22.4 (HOOCCH_2), 36.5 (NCH$_2$CH$_2$-CO), 123.2-132.2 (Ar-C), 149.5 Ar-*C*-NH), 162.4.2 and 163.4 (*CO*NH*CO*), 172.9(*C*OOH);

Analysis: $C_{15}H_{10}N_2O_6$ (314.21 g mol^{-1}): Calc. (%): C-57.28, H 3.18, N 8.91. Found (%): C-57.30, H 3.24, N 9,02;

2.2. Synthesis of dendrimer D1

PAMAM dendrimer (0.129 g, 0.25 mmol) and (0.314 g, 1.0 mmol) of NI1 were dissolved in 10 ml of *N,N*-dimethylformamide. The solution was stirred at 25 °C and after 24 hours, the product has been isolated by solvent evaporation in vacuum. The dendrimer obtained was washed with hexane and dried at air. Yield: 0.301 g, 76 %.

FT-IR (KBr) cm^{-1}: 1739, 1688, 1634, 1579, 1527, 1306, 1239, 1101, 823, 786, 756.

^{1}H NMR (DMSO) δ (ppm): 2.42 (d. $J = 7.1$ Hz, 8H, CH_2-CO), 3.10 (q. $J = 5.2$ Hz, 8H, HOOCCH2-), 2.68 (d. $J = 7.3$ Hz, 8H, CH_2-N-CH$_2$), 3.18 (d. $J = 7.2$ Hz, 4H, N-CH$_2$CH$_2$-N), 3.32 (s. 8H, HNCH$_2$-) 3.65 (d. $J = 6.8$ Hz, 8H, N-CH$_2$CH$_2$-NH), 4.10 (d. $J = 7.2$ Hz, 8H, CON-CH$_2$), 6.96 (bs, 4H, NH) 7.38 (t, $J = 7.8$ Hz, 4H, Ar-H), 7.89 (d, $J = 8.2$ Hz, 4H, Ar-H), 8.20 (d, $J = 8.5$ Hz, 4H, Ar-H), 8.48 (t, $J = 6.9$ Hz, 8H, Ar-H), 8.64 (d, $J = 8.2$ Hz, 4H, Ar-H), 9.42 (bs, 4H NHCO), 11.23 (s. 4H COOH); ^{13}C-NMR ($CDCl_3$): δ (ppm) = 30.8 (HOOCCH_2), 33.2 (NCH2CH$_2$-CO), 34.6

Table 1
Photophysical characteristics of compound D1 in organic solvents (see text).

	λ_A nm	ε l mol $^{-1}$ cm^{-1}	λ_F nm	ν_A - ν_F cm^{-1}	Φ_F
Acetonitrile	426	42 500	522	4 317	0.329
N,N-dimethylformamide	421	43 900	518	4 448	0.365
Ethanol	428	42 700	533	4 603	0.123
Methanol	430	42 800	538	4668	0.156
Chloroform	412	43 900	513	4 778	0.320
Dichloromethane	414	43 200	511	4 587	0.395
Tetrahydrofuran	414	43 300	509	4 508	0.339
Water (pH = 7.2)	422	41 800	520	4 466	0.114

(NH*C*H$_2$CH$_2$NHCO), 42.2 ((OC)$_2$N*CH$_2$*), 46.3 (NHCH$_2$*C*H$_2$NHCO), 51.2 (N*CH2CH2*N), 54.9 (N*CH$_2$*CH$_2$CO), 102.2-134.0 (Ar-C), 153.1 Ar-*C*-NH), 163.3 and 164.0 (*CO*NHCO), 170.3(COOH); 173.8 (*CO*NH).

Analysis: $C_{82}H_{84}N_{14}O_{20}$ (1584.56 g mol^{-1}): Calc. (%): C-62.10, H 5.30, N 12.36. Found (%): C-62.31, H 5.19, N 12,39;

2.3. Treatment of cotton fabric with NI1 or D1

To the dissolved in 5 ml ethanol-water 1:4 (v/v) solution 5 mg of D1 or NI1 have been added (1 g) cotton fabric (weight 140 g/m^2) at 40 °C and has been immersed in this solution for 60 min. After that, the cotton sample was washed with water and dried.

3. Results and discussion

Amphyphilic dendrimers with carboxyl groups in their peripheries show good antibacterial activity against gram-positive bacteria with low eukaryotic toxicity [13]. In previous studies, we have shown that cationic dendrimers [39] or neutral dendrimers and their metal complexes [29–32] exhibit microbiological activity. In this regard, we performed a targeted design to obtain a PAMAM dendrimer modified with 1,8-naphthalimides, in which a carboxyl group was previously introduced. In the design of D1, we assumed that this dendrimer would show good antibacterial properties with low toxicity, which is important in its subsequent use to obtain wound dressings.

Scheme 1 shows the synthetic route for the preparation of dendrimer D1 and its precursor HI1.3-(6-Nitro-1,3-dioxo-1H-benzo[de]isoquinolin-2(3 H)-yl)propanoic acid (NI1) has been obtained by reacting of 4-nitronaphthalic anhydride with β-alanine in 2-methoxyethanol solution, at 80 °C for 6 h according to the method described by Shaki et al. [40]. This product has been used to modify a poly(amidoanine) (PAMAM) dendrimer from zero generation containing four primary amino groups, in DMF solution at room temperature for 24 h [41]. Under these conditions, only a nucleophilic replacement of the nitro group from HI1 with an amino group from PAMAM structure is possible. The carboxyl group has not involved in a chemical reaction to form an imide group. The progress of the reaction was monitored by thin-layer chromatography in a 1: 1 n-heptane-acetone elution system until NI1 was exhausted. In this case, as a high molecular weight product, D1 remained at the start as a yellow fluorescent spot. The final product D1 has been isolated by pouring the reaction mixture into water, filtering the precipitate and washing with water.

3.1. Photophysical characteristics of D1

Compound NI1 contains an electron-acceptor nitro group at the C-4 position of the chromophore system, resulting in an absorption maximum in the ultraviolet region at 344 nm in DMF solution. The dipole moment of NI1 has been calculated to be 1.58 D. As a result of the covalent binding of NI1 to the periphery of the PAMAM dendrimer, the nitro group has been replaced by a secondary amino group. This altered the polarization of the chromophore system of the 1,8-naphthalimide fragments (D1 (branch) has a dipole moment of 9.00 D) and

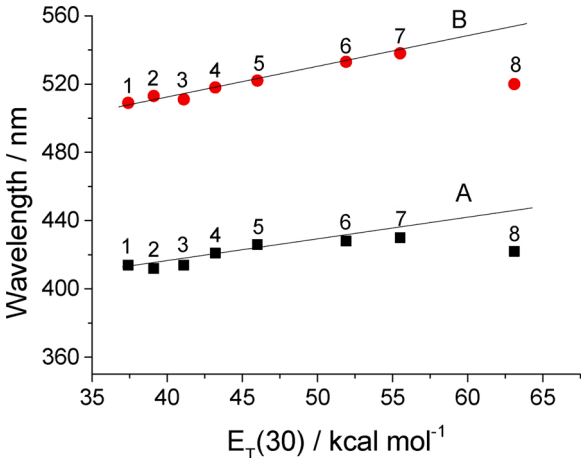


Fig. 1. Dependence of absorption (A) and fluorescence (B) maxima of dendrimer D1 on the solvent polarity: 1) chloroform; 2) dichloromethane; 3) tetrahydrofuran; 4) *N,N*-dimethylformamide; 5) acetonitrile; 6) ethanol; 7) methanol; 8) H_2O (pH = 7.2).

Table 2
TDDFT/B3LYP calculated λ_{max} and *f* (oscillator strength) in chloroform and methanol (solvents of different polarity).

Compound	Solvent	ε, D	λ_{max}, nm	*f*
D1(branch)	Chloroform	4.7	411	0.33
	Methanol	32.6	422	0.32

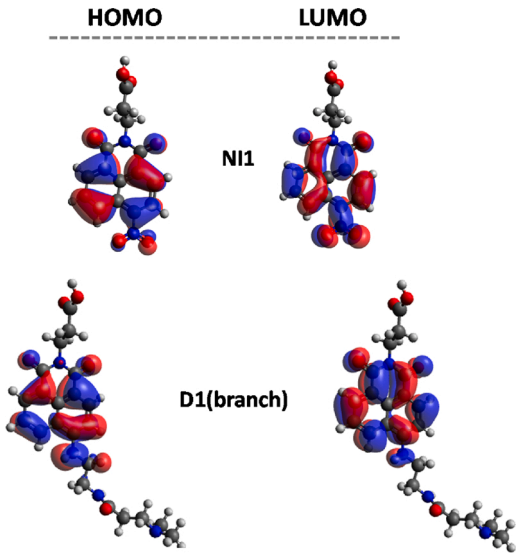


Fig. 2. Pictorial illustration of the highest occupied and lowest unoccupied molecular orbitals (HOMO and LUMO) ;isodensity plot, isovalue = 0.02 a.u.

dendrimer D1 absorbs in the visible spectral region at λ_A = 412-430 nm (Table 1). As a result of the polarization of the 1,8-naphthalimide structure, the dendrimer D1 emits yellow-green fluorescence with maxima in the spectral range λ_F = 509-538 nm. From Fig. 1 it can be seen that the absorption and fluorescence maxima of D1 depend on the polarity of the organic solvents. In both cases, they are bathochromatically shifted in the transition from nonpolar to polar medium, which means that the D1 dendrimer displays a positive solvatochromism. The Stokes shift (ν_A - ν_F), which is the difference between the absorbance and the fluorescence maxima of the fluorescent compounds, are at the range 4317-4778 cm^{-1}. The photophysical characteristics of the investigated

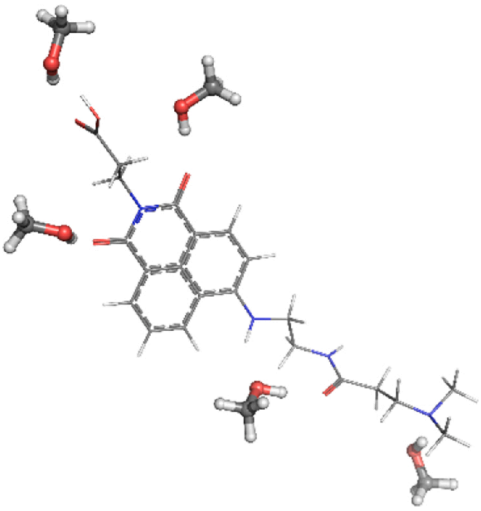


Fig. 3. Possible solute–solvent interactions of D1(branch) system as a hydrogen bond acceptor in polar protic solvent (methanol).

dendrimer are similar to those previously obtained by us dendrimers which have a secondary amino group as a substituent in the 1,8-naphthalimide structure [10,21].

TDB3LYP/6–31+G(d,p)//B3LYP/6-31+G(d,p) calculations gave the electronic vertical excitations of the studied D1 dendrimer in chloroform and methanol. D1 dendrimer was represented in a simplified manner by using a model with one branch of D1 and ethylenediamine core represented by a $N(CH_3)_2$ group (Figure S1, Supporting Information). solvatochromism (Table 2). The agreement between theory and UV experimental data is excellent. A small neglectable) systematic overestimation of the excitation energies (, typical for TDDFT calculations is observed for D1: 0.01 eV in chloroform and 0.05 eV in methanol medium.

The D1(branch) first excited state is determined by HOMO–LUMO transition with oscillator strengths $f = 0.32/0.33$. The frontier orbitals of NI1 and D1(branch) systems are shown in Fig. 2. There is no difference between the spatial distribution of the frontier orbitals - HOMOs and LUMOs are delocalized on the 1,8-naphtalimide core for both compounds (Fig. 2).

Quantum yield of fluorescence (Φ_F) provides information about the quantum efficiency of the dendrimer D1 which has been measured using a relative method. The Φ_F of D1 was calculated by comparing its fluorescence intensity to Rhodamine 6 G uzing as reference sample.

$$\Phi_F = \Phi_{st}\frac{S_u}{S_{st}}\frac{A_{st}}{A_u}\frac{n_{Du}^2}{n_{Dst}^2} \quad (1)$$

where Φ_{st} is the quantum yield of the standard (Rhodamine 6 G $\Phi st = 0.94$);

A_{st} and A_u are absorbance of the standard and sample;

S_{st} and S_u are the integrated emission band areas of the standard and sample,

nD_{st} and nD_u are the solvent refractive indexes of the standard and sample;

It is apparent from the data in Table 1 that the polarity of the organic solvents does not significantly affect the Φ_F. The results have shown that the two to three times lower quantum yield was obtained in polar protic solvents (alcohols and water solutions). These solvents contain hydroxyl groups through which hydrogen bonds can be formed with the 1,8-naphthalimide chromophore system. For example, effective hydrogen bonds can be formed by the carbonyl (C=O) imide structure, resulting in a change in the polarity of the chromophore system. Hydrogen bonds formation is also possible with participation of the amide groups and tertiary nitrogen atoms of the PAMAM structure, which probably alters

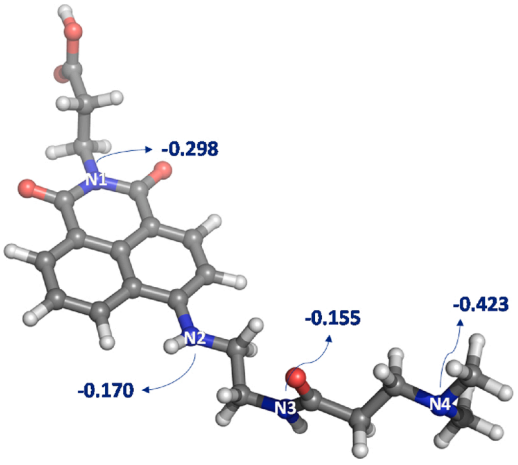


Fig. 4. Possible protonation sites in the molecular structure of D1(branch) and Hirshfeld charges on the nitrogen atoms.

Table 3
Gibbs free energy differences (ΔG) between the protonated forms of the model D1(branch) fragment (relative stabilities) of the protonated D1(branch) forms in the gas phase (ΔG^1) and in water (ΔG^{78}).

Structure	ΔG^1, kcal mol-1	ΔG^{78}, kcal mol^{-1}
D1(branch)-H^+ (N1)	25.97	44.52
D1(branch)-H^+ (N2)	2.89	17.16
D1(branch)-H^+ (N3)	31.36	30.87
D1(branch)-H^+ (N4)	0.00	0.00

the 3-D structure of the dendrimer molecule and as a result of this change the quantum yield decreases. DFT predicted solute–solvent interactions for D1 (branch and core fragments) in a hydrogen bond donor solvent (MeOH) are visualized in Fig. 3.

3.2. Dependence of the fluorescence intensity of D1 on the pH

pH value is a critical parameter in numerous fields, including chemical and food industry, pharmacy, medical and biological science, biochemical process, *etc.* [42]. 1,8-Naphthalimides are one of the widely used chromophore systems for pH determination. In most of the 1, 8-naphthalimide derivatives, in which the fluorescence emission is dependent on the pH values of the medium, the intensity of the emitted fluorescence is higher in acidic media. This can be explained by the

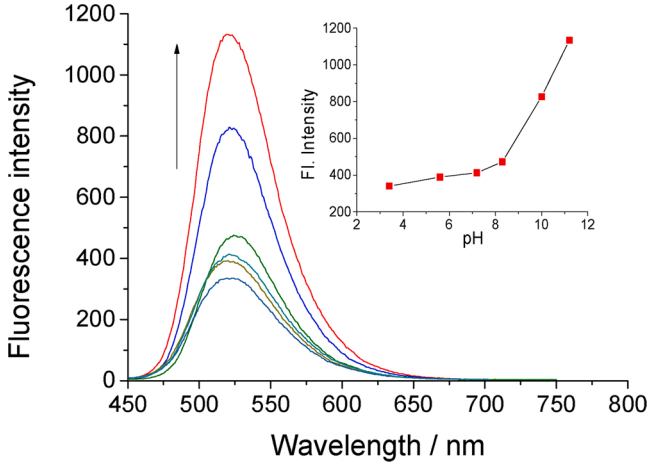


Fig. 5. Fluorescence spectra and dependence of the fluorescence intensity of D1 on the pH in aqueous solution.

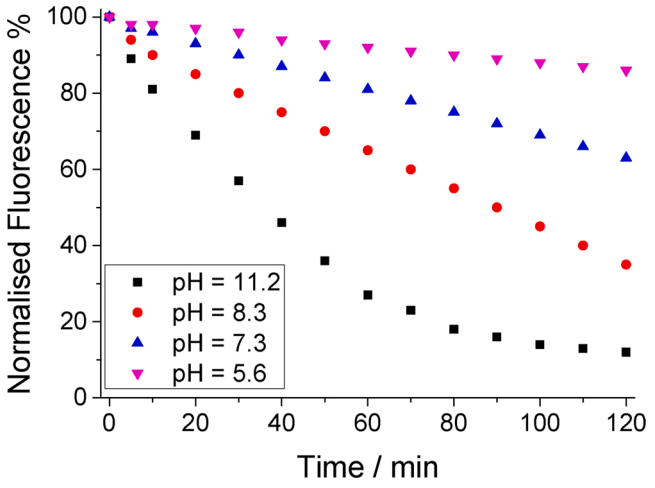


Fig. 6. Release of the dendrimer D1 from the cotton surface at different pH values.

Table 4
The amount at % of NI1 or D1 released from cotton fabric in aqueous solution at different pH values.

	pH =5.6	pH =7.3	pH =8.3	pH =11.2
NI1 %	12	30	70	99
D1 %	10	25	65	90

protonation of amino groups, which are most commonly linked to the C-4 atom of the chromophore system, and the stop of the photoinduced electron transfer and the restoration of fluorescence intensity [43]. In some structures, such as deprotonated hydroxyl group bonded directly to the naphthalene nucleus of 1,8-naphthalimide, the fluorescence is enhanced in an alkaline medium [44].

To probe the dominant protonation sites on the D1 dendrimer structure, we compared the stabilities of the different protonated forms of the model D1(branch) fragment (Fig. 4 and Table 3). D1(branch)-H^+ (N4) form appears to be the most stable one. Hirshfeld atomic charges obtained for the optimized D1(branch) geometry also show that the protonation preferably occurs at the ethylenediamine core (represented by a $N(CH_3)_2$ group in our model).

The pH-dependent fluorescence intensity of dendrimer D1 was investigated (Fig. 5). The fluorescence intensity increases with increasing pH values of the solution. In an acidic medium, D1 emits a low fluorescence, which is enhanced in an alkaline environment (pH > 9). This indicates that the fluorescence emission of this dendrimer is low in an acidic environment, which was not observed in neutral

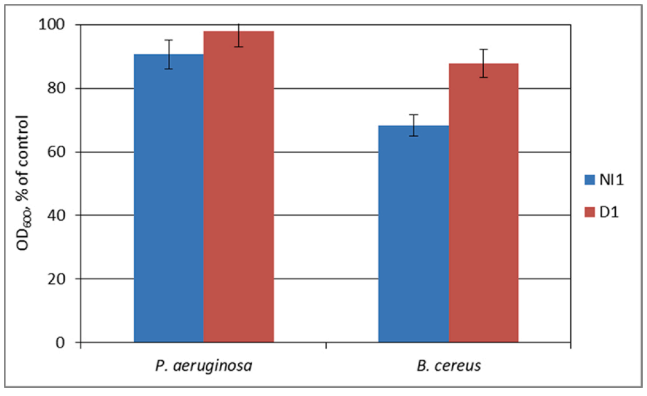


Fig. 7. Reduction of the growth of model bacteria by cotton fabrics, treated with NI1 and D1.

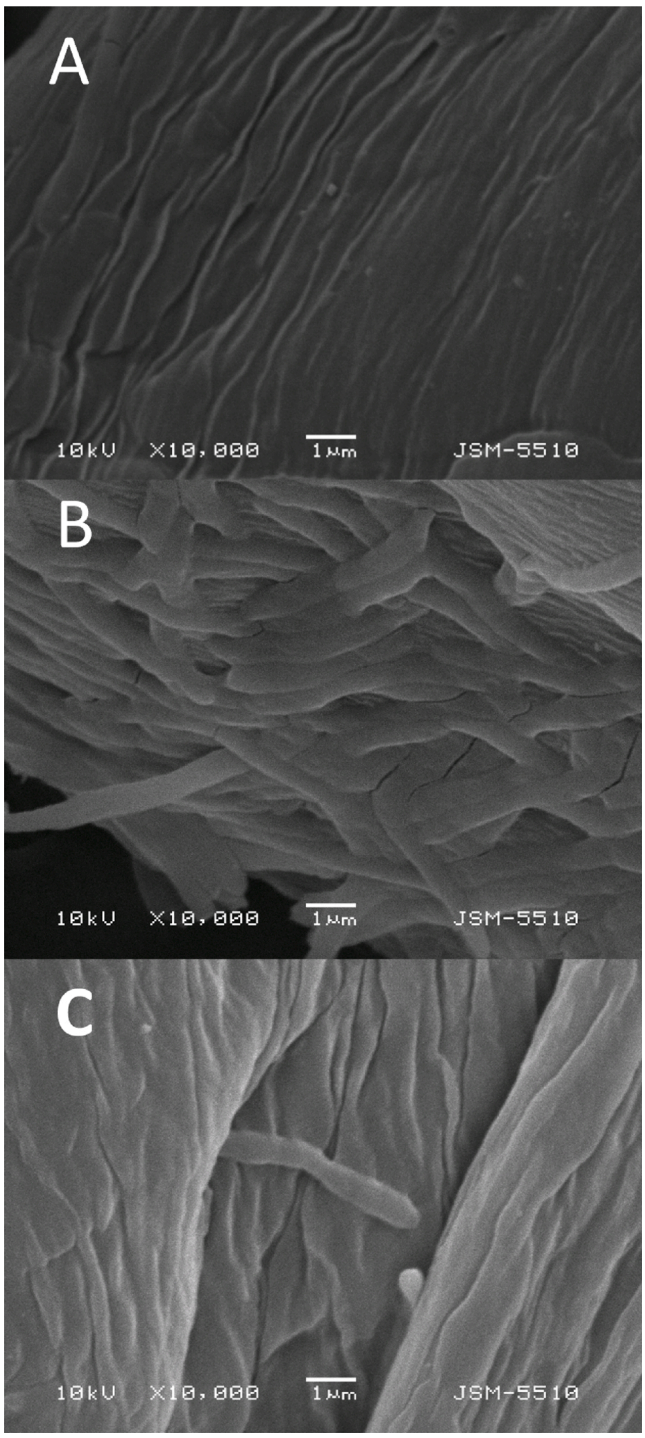


Fig. 8. SEM micrographs of cotton fabric tested against *B. cereus* at 10000x magnification: A) – initial cotton fabric before treatments; B) - bacterial biofilm on cotton fabric before treatment with D1; C) - inhibition of biofilm formation on cotton fabric treated with D1.

dendrimer molecules [10,23,24].

In order to obtain an antibacterial textile material, dendrimer D1 has been deposited on cotton fabric. The amount of the deposited dendrimer on the surface of the fabric has been examined spectrophotometrically using absorption spectroscopy. Approximately 85% of the dendrimer initially used (0.5 w%) has been fixed to the cotton fabric. Release of the dendrimer from the surface of the cotton fabric has been investigated at 37 °C and different pH for 2 hours. The results obtained at four pH values: pH = 5.6, 7.3, 8.3 and 11.2 are shown in Fig. 6. It is seen that in

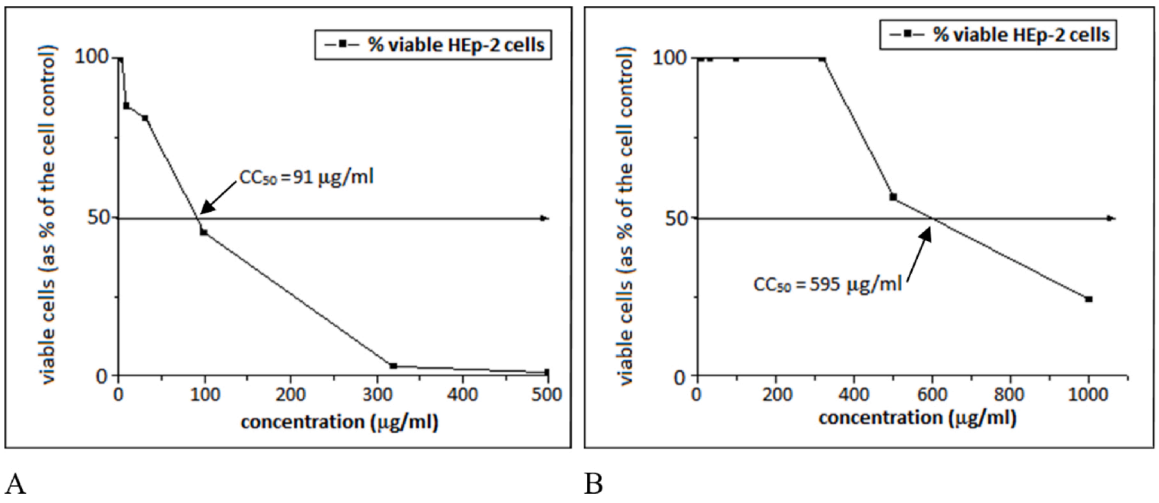


Fig. 9. Cytotoxicity values of the compounds NI1 (A) and D1 (B) towards HEp-2 cells.

acidic medium (pH = 5.6) about 10% of the deposited D1 is separated from the surface of the fabric. At neutral pH = 7.3, 25% of dendrimer has been released, and in a slightly alkaline medium (pH = 8.3) 65% of the dendrimer was separated from the cotton fabric. At these pH values, the separation follows a linear relationship. The dendrimer is most rapidly detached in a highly alkaline medium (pH = 11.2) in which the carboxyl groups are deprotonated and the solubility correspondingly higher. In this case, linear dependence is observed in the first 60 min (about 70% separation), after which the process slows down in the next hour, with almost 90% of the dendrimer being separated at the end of the second hour. This indicates that the pH of the medium has a significant effect on the retention of the dendrimer on the surface of the cotton fabric. A similar dependence has been observed in the study of the release of NI1 from the textile surface. From Table 4 it can be seen that in the case of NI1 the amount is slightly greater and in the highly alkaline medium almost the whole amount has released from the cotton fabric. This difference is probably due to the larger chemical structure of the dendrimer compared to NI1 and hence to the possibility of forming more hydrogen bonds and, therefore, to its stable fixation to the cotton fabric.

3.3. Antimicrobial activity

In the agar diffusion test, no inhibition zones were observed by both compounds against the model strains. In MPB, antimicrobial activity of the compounds was tested quantitatively against the tested bacterial strains *B. cereus* and *P. aeruginosa*. The results showed that Gram-positive B. cereus exhibited lower resistance to the compounds compared to Gram-negative P. aeruginosa. NI1 was found a little m ore toxic to the tested strains than D1. NI1 inhibited completely the growth of *B. cereus* at concentration of 240 µg/ml, while D1 inhibited more than 95% of the growth of the strain at 450 µg/ml. Gram-negative P. aeruginosa demonstrated higher resistance towards the compounds. At the highest test concentration of 600 µg/ml, about 64% growth inhibition of *P. aeruginosa* was established by NI1, and no inhibition was observed by D1. The difference in susceptibility of Gram-positive and Gram-negative bacteria can be explained by differences in the structure of the bacterial cell walls [45].

3.4. Antibacterial activity of modified cotton fabrics

At the contact of pathogenic microorganisms with the hydrophilic cotton surface, especially in humid environments, may result in bacterial biofilm formation. Preventing the formation of such a biofilm is particularly important in wound dressings where they are in direct contact with human skin or injured areas. In these cases, the prevention of bacterial infections is of particular importance. Antimicrobial modification of cotton surfaces is an alternative way of preventing the formation of highly resistant biofilms and can be achieved by various methods [46]. The antimicrobial effect of cotton fabrics modified by NI1 and D1 was evaluated by growth reduction of Gram-positive *B. cereus* and Gram-negative *P. aeruginosa*.

The results showed that the cotton material treated with NI1 reduced the growth of *B. cereus* by about 32% while the D1 treated sample was less active reducing the growth by about 12% (Fig. 7). Against *P. aeruginosa*, lower microbial reduction by NI1 and D1 was observed (about 10% and 2%, respectively). It could suggest that both slow diffusion of dendrimers from the cotton textile into the medium, and direct contact with bacterial cells contributed to the antibacterial effect of treated cotton fabrics. Several factors may affect considerably the antimicrobial effect of textiles as reported by Surdu et al. [47].

It was of interest to examine the cotton surface for bacterial biofilm formation by SEM technique. Fig. 8 shows SEM micrographs of cotton fabric treated with D1 before and after 24 hours of contact with *B. cereus*. Cotton fabric treated with D1 and untreated with bacteria has been shown in Fig. 8A. Fig. 8B shows the formation of a stable biofilm on the cotton fabric before treatment with D1. Applying dendrimer D1 to the fabric significantly reduces the adhesion of bacteria and biofilm formation. Only single bacteria have been detected on the fabric surface (Fig. 8C). This means that dendrimer D1 prevents the retention of bacteria on the surface of the cotton tissue and from there to the formation of a bacterial biofilm. Therefore, D1 has the potential to be used for the preparation of antibacterial textile. Similar protective properties were observed using 1,8-naphthalimide modified PAMAM dendrimers [29–31].

3.5. Cytotoxicity

For the preperation of wound dressings it is important to investigate the citotoxicity of dendrimer D1 and 1,8-naphthalimide NI1. The evaluation of cytotoxicity of antimicrobials is a critical step to guarantee their safe use. The results for the cytotoxicity of the compounds are shown in Fig. 9. It has been found that the newly synthesized dendrimer D1 is considerably less toxic (about 6.5 fold) to the HEp-2 cell line compared to the monomer NI1 which can be considered as a great advantage of the dendrimer over the corresponding monomer. This confirms the effect observed by Grinstaff et al. that amphiphilic anionic dendrimers exhibit a good antibacterial effect with minimal toxicity [13]. Also dendrimer D1 was found to show lower cytotoxicity to similar PAMAM dendrimers and hyperbranched polymers modified with 1, 8-naphthalimides or acridine [25,31,33].

4. Conclusion

A new zero-generation anionic PAMAM dendrimer modified with four 1.8-naphthalimide fragments has been synthesized. Its photophysical characteristics were investigated in organic solvents of different polarity and the dendrimer was found to have yellow colour and emits yellow-green fluorescence. The position of the absorption and fluorescence maxima depends on the polarity of the medium. The dendrimer and its monomeric structural analogue were deposited on a cotton fabric. The release of both compounds from the surface of the cotton fabric has been evaluated spectrophotometrically, and it was found that this process is faster in a highly alkaline medium. The antimicrobial activity of the compounds has been tested in agar, in solution, and after their deposition on cotton cloth. Both compounds have been found to have higher activity against the tested Gram-positive bacteria than to Gram-negative bacteria. The dendrimer D1 has been found to be considerably less toxic (about 6.5 fold) to the HEp-2 cell line compared to the monomeric anionic analogue NI1. This indicates that the dendrimer has higher potential to be applied as antimicrobial agent. Deposited on a cotton fabric, it prevents the formation of bacterial films and can be used for preparation of wound dressing.

CRediT authorship contribution statement

Desislava Staneva: Investigation, Methodology, Writing - review & editing. **Silvia Angelova:** Methodology, Investigation. **Evgenia Vasileva-Tonkova:** Investigation. **Peter Grozdanov:** Investigation. **Ivanka Nikolova:** Investigation. **Ivo Grabchev:** Supervision, Funding acquisition, Conceptualization, Methodology, Writing - original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was particularly supported by Grants N№ KOST-KII-19, Fund “Scientific Research”, Ministry of Education and Science of Bulgaria. S.A. acknowledges the provided access to the e-infrastructure of the NCHDC – part of the Bulgarian National Roadmap for RIs.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jphotochem.2020.112878.

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