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Research Article

Spectral and Antimicrobial Activity of Picric Acid Charge-Transfer Complex With Modified Poly(Propylene Amine) Dendrimer

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Abstract

The charge transfer complex of poly(propylene amine) dendrimer peripherally modified with 1,8-naphthalimide units from second generation (as donor) and picric acid (as acceptor) has been synthesized and characterized by elemental analysis, IR and ^{1}H-NMR-spectroscopy. Its basic photophysical characteristics have been investigated in organic solvents with different polarity. The electronic absorption spectra indicated that the charge transfer complex was formed with proton migration from the acceptor to the donor followed by hydrogen bonding via N+–H···O–. The stoichiometry of the dendrimer charge transfer complex has been investigated by fluorescence spectroscopy and it was found to be 4:1 [PPA8$(PA)_4$]. The complex was found to inhibit the growth of various Gram-positive and Gram-negative bacteria and yeasts.

Keywords: 1,8-Naphthalimide; Picric Acid; Dendrimer; Antibacterial Activity; Infrared Spectra; Fluorescence

Introduction

Dendrimers are a relatively new, hyperbranched and monodisperse class of polymers, with well-defined molecular structure Dendrimers combine the photophysical properties of low- and high molecular weight substances [1]. A great number of the same or different functional groups are located both in the branches and in the periphery, which gives many opportunities for target modification of their properties [2-6]. Functionalising the dendrimers with photoactive groups expands the spheres of their applications [7,8]. Periphery modified dendrimers comprise many closely located chromophores which could be independent from each other or can interact. In the latter case the dendrimers acquire new properties defined as the "dendrimer effect"[9]. Poly(propylene amine) (PPA) and polyamidoamine (PAMAM) are two commercial classes of dendrimers with particular application in various areas [1,9,10]. They are water-soluble, non-immunogenic and biocompatible.

Dendrimers might enhance the solubility of lipophilic drugs due to hydrophobic interactions, hydrogen bonding or electrostatic interaction between surface functional groups of the dendrimer and drug. Moreover, some of dendrimers show antibacterial and antifungal activity [11] and provide the opportunity for complex therapy in which the dendrimers are not only the drug carrier but also an adjunctive component of the dosage form [12].

In recent years, charge-transfer (CT) complexes of different organic compounds have been studied intensively due to their

special type of interactions, accompanied by transferring of electrons from the donors to the acceptors. Charge transfer complexes play a central role in bactericides, fungicides, insecticides and various light-driven physical and chemical processes [13-15]. Picric acid (PA) is a good electron acceptor and is known to form stable colored charge transfer complexes with many donors [16-18]. During the electron transfer this acidic acceptor forms an ion pair adduct [19]. CT complexes are appealing also as potential antimicrobial agents. For example, CT complexes of a drug molecule may absorb in the visible range and thus lead to easy detection and estimation of the drug [20]. Nowadays, there is a need for new antimicrobials due to rise of resistance in many common bacterial pathogens [21]. By studying the antimicrobial activity of CT complexes new types of antimicrobial agents could be developed.

In this paper we describe the charge-transfer interaction between peripherally modified poly(propylene amine) dendrimer from second generation (as donor) and picric acid (as acceptor). The stoichiometry of the reaction, IR and photophysical characterization of the obtained CT complex was discussed. In addition, the antimicrobial activity of the complex was investigated.

Experimental part

Materials and methods

The synthesis and basic photophysical characteristic of PPA8 dendrimer from second generation modified with 1,8-naphthalimide units were described earlier [22]. Infrared analysis was carried out using Infrared Fourier transform spectrometer (IRAffinity-1 "Shimadzu") with the diffuse-reflectance attachment (MIRacle Attenuated Total Reflectance Attachment) at a 2 cm^{-1} resolution.All IR spectra were recorded in solid state. UV/vis spectrophotometric investigations were performed using "Thermo Spectronic Unicam UV 500" spectrophotometer. The fluorescence spectra were taken on a "Cary Eclipse" spectrophotometer. All spectra were recorded at concentrations of $1x10^{-6}$ mol dm–3 using 1 cm path length synthetic quartz glass cells. Organic solvents used in this study (methanol, ethanol, 2-propanol, tetrahydrofuran and a dichlorethane) were of spectroscopic grade, and they were used as obtained from Sigma-Aldrich. Fluorescence quantum yield was determined on the basis of the absorption and fluorescence spectra using Coumarin 6 as a reference (Φ_{st} = 0.78 in ethanol) [23]. ^1H (600.13 MHz) spectrum was performed on an AVANCE AV600 II+NMR spectrometer. The measurements were carried out in $CDCl_3$ solution at ambient temperature. The chemical shifts were referenced to a tetramethylsilane (TMS) standard.

Synthesis of the solid charge transfer complex of picric acid with modified poly(propileneamine) dendrimers from second generation [PPA8(PA)$_4$]

The solid charge transfer complex of picric acid with PPA8 from second generation was synthesized by reaction of PPA8 (0.29g, 1 mmol) dissolved in 10 ml chloroform with picric acid (0.13g, 6 mmol). The mixture was stirred at 400C for 60 min. The precipitate was filtrated, washed three times with chloroform (3x10 ml), and then dried under vacuum at 40^0C.

Yield: 86%

^{1}H-NMR (CDCl3, 600MHz ppm): 9.12 (s, 8H, Ar-PA, 7.62 (dd, J=5.86 Hz, J=5.81 Hz, 16H, ArH), 7.45 (dd, J=5.88 Hz, J=5.8 4 Hz, 16H, ArH), 7.24 (m, 16H, ArH), 4.14 (q, 16H, (OC)2NCH2), 1-70-1.54 (m, 4H, NH+) 1.44-1.01(m, 32H, CH2N< + 4H, >NCH-2CH2CH2CH2N<), 0.97-0.64 (m, 24H, >NCH2CH2CH2N< + 4H, >NCH2CH2CH2CH2N<).

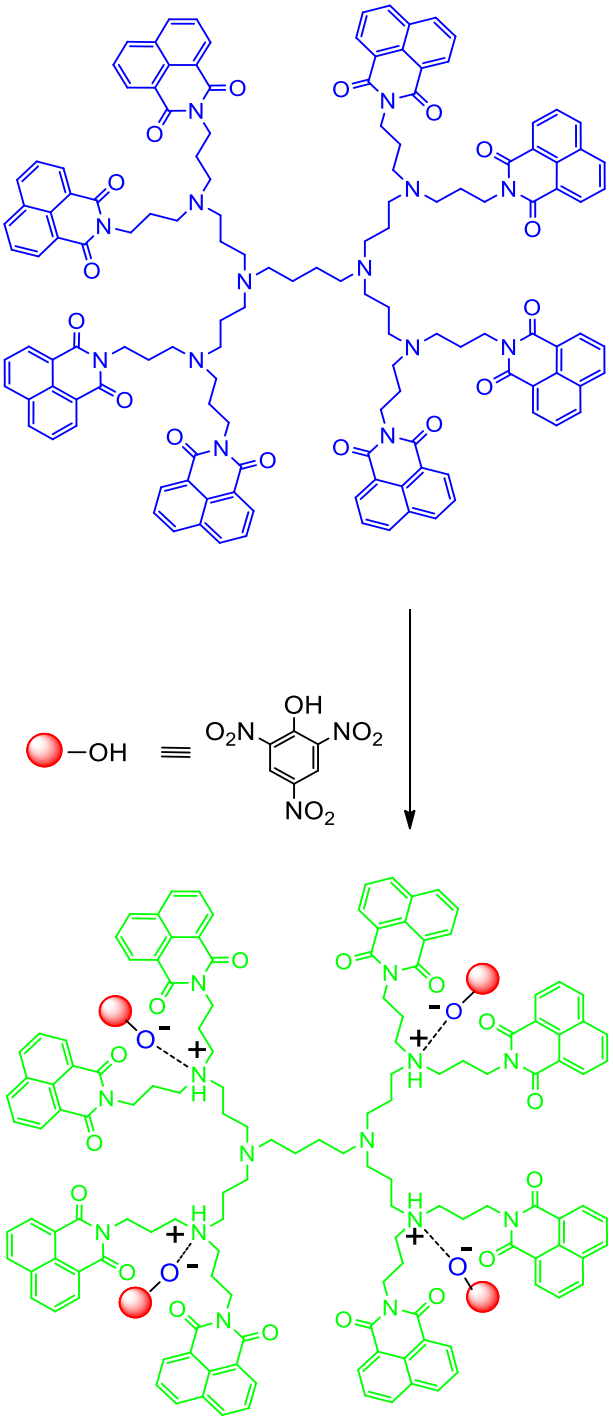
Analysis: $C_{160}H_{140}N_{26}O_{44}$ (3128.3): Calcd. C 61.37, H 4.47, N 11.63; Found C 61.49, H 4.59, N 11.79.

Antimicrobial assay

The antimicrobial potential of the newly synthesized complex [PPA8(PA)$_4$] was evaluated *in vitro* for its antibacterial and antifungal activities using disc agar-diffusion method [24]. The antibacterial activity was tested against Gram-positive bacteria *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 11778, *Sarcina lutea* ATCC 9341 and *Micrococcus luteus* ATCC 9631, and Gram-negative bacteria *Pseudomonas aeruginosa* NBIMCC 1390 (National Bank of Industrial Microorganisms and Cell Cultures, Sofia, Bulgaria), *Escherichia coli* JM105 5α, *Acinetobacter johnsonii* ATCC 17909 and *Xanthomonas* oryzae ATCC 35933. The antifungal activity was tested against the yeasts *Saccharomyces cerevisiae* ATCC 9763 and *Candida lipolytica* 76-18 (Institute of Microbiology collection, Sofia, Bulgaria). The CT complex was dissolved in DMSO in concentration 5 mg/ml and 20 µl of this solution was spotted on filter paper discs (6 mm in diameter) and applied onto nutrient agar plates with each freshly grown indicator culture. Standard discs of Gentamicin (antibacterial agent) and Nystatin (antifungal agent) were used as reference controls. Each treatment was performed in three replicates. After incubation of the plates for 24-48 h at 28 ± 2°C, the inhibition zone diameters (including disc), were measured.

Results and Discussion

Charge transfer complex [PPA8(PA)$_4$] formed from the interaction between PPA8 and picric acid was synthesized by the mixture of both compounds in chloroform solution at 40^0C for 60 minutes. After cooling to room temperature, the yellow precipitate was filtered and washed with chloroform (Scheme 1).



Scheme 1. Synthesis and chemical structure of [PPA8(PA)$_4$] charge transfer complex.

Photophysical characteristics of charge transfer complex [PPA8(PA)$_4$]

Initial PPA8 dendrimer absorbs at UV region with maximum at λ_A = 326 nm and emits low (Φ_F = 0.007) blue fluorescence emission with maximum at λ_F = 375nm. [22].

In Table 1 are summarized the basic photophysical characteristics of [PPA8(PA)$_4$] measured in organic solvents of different polarity. Charge transfer complex [PPA8(PA)$_4$] absorbs in the λ_A = 337-354 nm region (**π-π*** transition) and an additional maximum at λ_A = 400-422 nm (n-**π*** transition) and have an intensive yellow color due to the complex formation (Table 1 and Figure 1). The absorption spectra provides evidence for the existence of new bands of the charge transfer complex which indicate an interaction associated with proton migration from the picric acid acceptor to the PPA8 donor followed by intermolecular hydrogen bonding as presented in Scheme 1.

	2-propanol	Methanol	Ethanol	Tetrahydrofuran	Dichlorethane
λ_A (nm)	351 400	352 400	354 400	348 406	337 422
ε (l mol^{-1} cm^{-1})	83000	82900	82300	85800	85900
λ_F(nm)	518	525	524	515	511
ν_A -ν_F (cm^{-1})	9185	9361	9164	9318	10104
Φ_F	0.09	0.02	0.03	0.11	0.14

Table 1. Photophysical properties of CT complex [PPA8(PA)$_4$].

In all tested organic solvents the dendrimer complex emits yellow-green fluorescence with maxima at λ_F= 511-525 nm region, which is not typical for the free PPA8 dendrimer. This means that PPA8 dendrimer changes its fluorescence from blue to yellow-green after CT complex formation with PA. In this case, the yellow color of [PPA8(PA)$_4$] is due to the different polarization of 1,8-naphthalimide chromophoric system, compared to free PPA8 dendrimer. As seen from the data, [PPA8(PA)$_4$] has negative solvatochromism (Table 1). The Stokes shift (ν_A -ν_F) indicates the difference in the properties and structure of the [PPA8(PA)$_4$] in the ground state S_0 and the first exited state S_1. The Stokes shift values are very large and they are in the 9164-10104 cm^{-1} region which is approximately two fold higher than the PPA8 values [25]. This indicates a destabilising effect of the PA on the planarity of dendrimer molecule. The quantum yield of fluorescence (Φ_F) is relatively low in all organic solvents and is in the region of Φ_F= 0.02-0.14 but there is a tendency of enhancement.

Because the fluorescence spectroscopy is more sensitive compared to the absorption spectroscopy, we have used this technique to investigate the interaction of picric acid with PPA8 dendrimer. As seen in Scheme 1, there is only tertiary amino groups in the core of peripherally modified with 1,8-naphthalimide units PPA8 dendrimer, and these amino groups can act as a powerful electron donor. They can react with hydroxyl groups from picric acids and form hydrogen bonds $^{+}$N—H ·····O^{-}. The titration profile of dendrimer PPA8 with picric acid is plotted in Figure 2. It is seen than 4 mol of picric acid react with 1 mol of dendrimer to form charge transfer complex [PPA8(PA)$_4$] with stoichiometry 4:1.

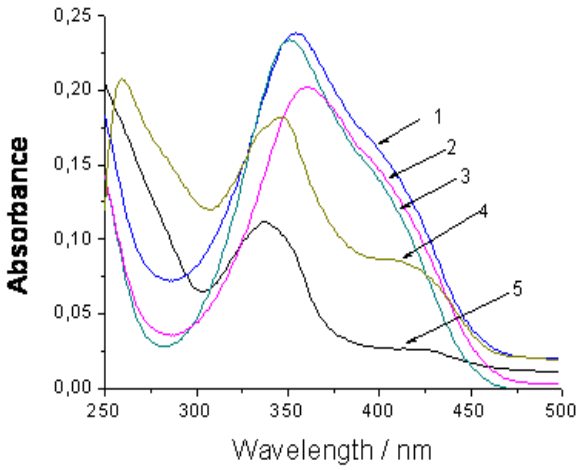


Figure 1. Absorption spectra of [PPA8(PA)$_4$] in : ethanol (1), methanol (2), 2-propanol (3), tetrahydrofurane (4), chloroform (5).

Infrared spectra of [PPA8(PA)$_4$] complex

Stretching and deformation vibrations of the main functions in the infrared region of the spectra of PPA8 dendrimer and [PPA8(PA)$_4$] are summarised in Table 2. As can be seen, the characteristic frequencies for the mains groups of PPA8 are approximately identical with these for [PPA8(PA)$_4$]. Similar results have been described recently [25].

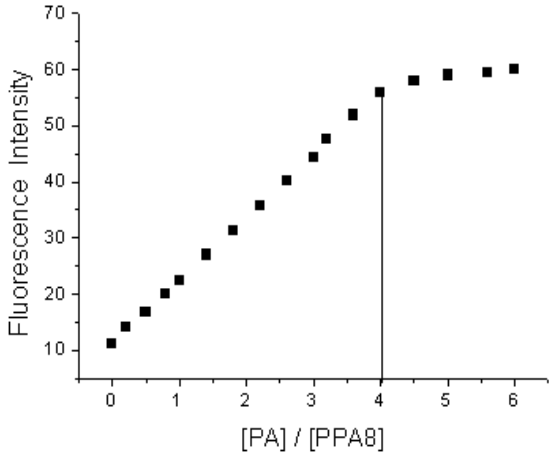


Figure 2. Fluorescence titration curve for [PPA8(PA)$_4$] in chloroform solution (PPA8 c= 1x10^{-6} mol dm^{-3}).

That means than the influence of picric acid is not so strong to cause a change in the frequency of oscillation of the characteristic groups. This is especially well seen in the region 1698 and -1655 cm^{-1}, where absorb both carbonyl groups ν(C=O) [26,27].

Figure 3 shows the difference in the spectra of PPA8 and its complex [PPA8(PA)$_4$]. The most significant difference is observed in the region 1200-1400 cm^{-1}, where are the characteristic bands of nitro (-NO$_2$) and C-N groups [28]. The asymmetric stretching vibration of the –NO$_2$ group is sensitive to the polar influences and the electronic states. In the spectrum of [PPA8(PA)$_4$] a specific doublet appears at 1564-1555 cm, and new intensive bands at 1313 cm^{-1} are characteristic to the nitro groups of picric acid. The C-N bond showed stretching vibration absorption bands at 1363, 1337,1262 and 844 cm^{-1}. C–NO$_2$ stretching vibration are observed at 1078 and 910 cm^{-1}. A broad doublet at the spectrum of [PPA8(PA)$_4$] has been detected with maximum at ca. 2646 cm^{-1} which was not observed at PPA8 spectrum. Probably It can be ascribed to the stretching vibrations of the picric acid hydrogen bonds ($^+$N—H ·····O$^-$). (Figure 4).

NMR spectral characterization of [PPA8(PA)$_4$] complex

The chemical structure of [PPA8(PA)$_4$] was confirmed by ^{1}H-NMR spectroscopy in chloroform. The singlet peak observed at δ 9.12 has been assigned to aromatic protons of the picrate moiety in the complex. The aromatic protons from 1,8-naphthalimide units are at δ(ppm) 7.62, δ 7.45 and δ 7.24. Aliphatic protons appear as multiplets in the areas of:δ (ppm) 4.14,δ 144-1.01 and δ 0.97-0.64. The multiplets at δ(ppm) 1.70-1.54 are due to the protonated nitrogen atoms (NH$^+$). The characteristic signals for aromatic and aliphatic protons of PPA8 dendrimer structure from the complex are shifted to the lower δscale as compared to the initial PPA8 dendrimer [22]. Also, the intensities of the aromatic signals were significantly affected by the complexation process and the accompanying changes in the structural configuration. The characteristic proton peaks from hydroxyl group of picric acid atδ = 11.94 ppm, [29], was absent in the spectrum of the [PPA8(-PA)$_4$] complex.

	PPA8 (cm^{-1})	[PPA8(PA)$_4$] (cm^{-1})
ν_{C-H} arom	3068	3073
ν_{CH2} aliph	2958 2874	2972 2866
$\nu^{AS}_{C=O}$	1696	1698
$\nu^{S}_{C=O}$	1654	1655
$\nu_{C=C}$ arom	1624 1595	1609 1589
ν_{CH2} aliph	1437	1436
ν_{CNC}	1350 1173	1362 1164
δ_{C-H} arom	844 774	844 774

Table 2. Infrared spectra of 4PPA8 and its complex [PPA8(PA)$_4$].

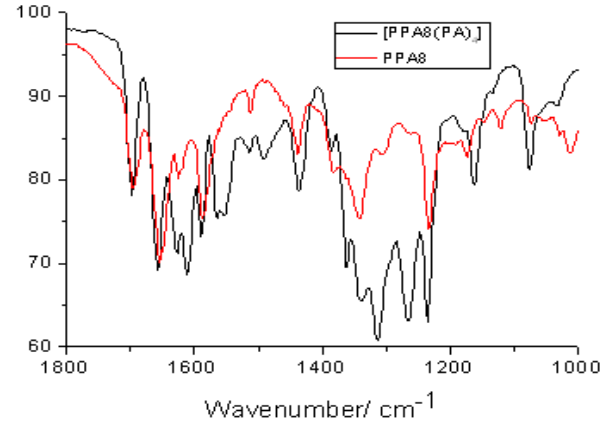


Figure 3. FTIR spectra of PPA8 and [PPA8(PA)$_4$]

Microbiological investigations

The antibacterial activity of the newly synthesized complex was tested against eight bacterial strains, and the antifungal activity against two yeast strains. The [PPA8(PA)$_4$] complex (100 µg/disc) demonstrated inhibitory activity against the growth of six of the test bacteria with zones of inhibition in the range 10-12 mm (Figure 5). In comparison with standard drug, the complex exhibited lower or comparable activity. Gram-negative strains E. coli and P. *aeruginosa* were resistant towards the studied complex. The complex exerted good antifungal activity against the test yeasts, which were found resistant towards the control antifungal agent nystatin. Therefore, the new dendrimer complex has potential in developing new antimicrobial agents.

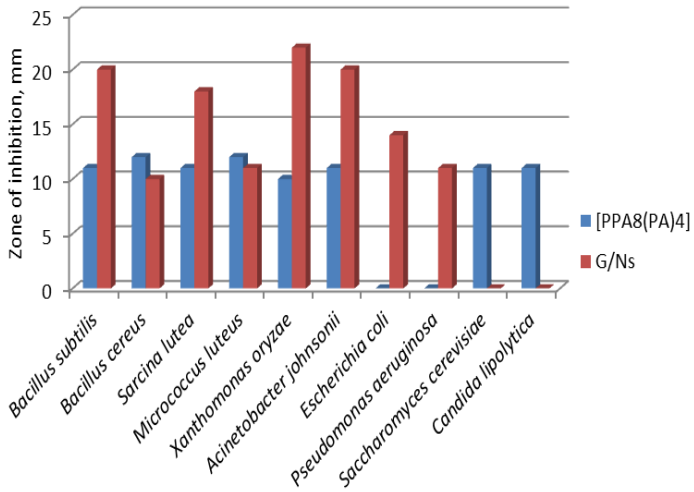


Figure 4. FTIR spectra of PPA8 and [PPA8(PA)$_4$].

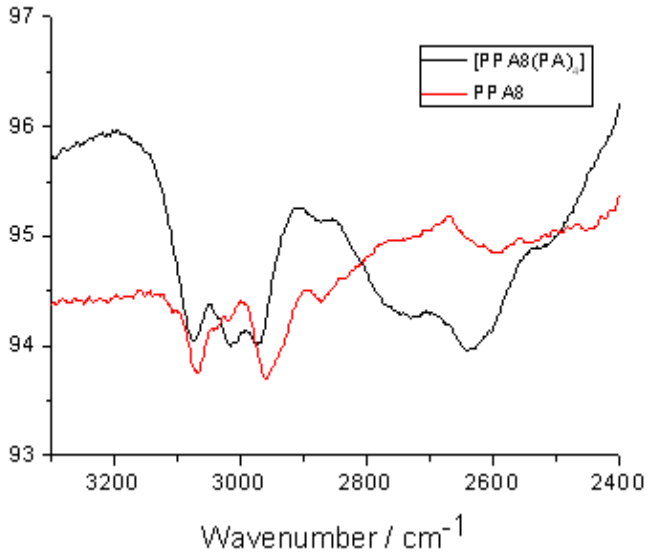


Figure 5. Inhibition of the growth of some model bacteria and yeasts by [PPA8(PA)$_4$] complex. G/Ns, gentamicin/nystatin used as a standard antibacterial/antifungal agent.

Conclusion

Fluorescence charge transfer complex [PPA8(PA)$_4$] from modified PPA dendrimers with 1,8-naphthalimide units and picric acid has been synthesized and characterized by electronic (absorption and fluorescence), FT-IR and ^{1}H-NMR spectroscopy. The data from elemental analysis, NMR and FT-IR spectroscopy indicated that the amine and phenolic groups are involved in the formation of the charge transfer complex between PPA8 and PA. The main photophysical characteristics have been investigated in organic solvents of different polarity and it was shown that [PPA8(PA)$_4$] dendrimer emits yellow-green fluorescence. The formation of charge transfer complex of PPA8 and PA was investigated spectrophotometrically in chloroform at room temperature by fluorescence spectroscopy and it was found a complex formation with 1:4 stoichiometry. The [PPA8(PA)$_4$] complex exhibited antibacterial and antifungal activity against various species of bacteria and yeasts. Investigation of the antimicrobial activity of such type of dendrimer complexes would expand the potential biological application of the dendrimers.

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