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Synthesis and photophysical properties of 1,8-naphthalimide-labelled PAMAM as PET sensors of protons and of transition metal ions

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Abstract

Novel fluorescent derivatives of generation 0 polyamidoamine (PAMAM), comprising 1,8-naphthalimide units have been synthesized. Their basic photophysical properties in organic solvents of different polarity have been determined and discussed. A 4-*N*,*N*-dimethylaminoethylene-1,8-naphthalimide, labelled PAMAM, has been synthesized with designed fluorescent intensity and high sensitiveness to the presence of protons or transition metal cations. This sensitiveness has been ascribed to a photo-induced electron transfer from the amino groups of the central PAMAM core and the terminal amino groups of dialkylamino moieties to the electron-deficient component in 1,8-naphthalimide. © 2002 Published by Elsevier Science Ltd.

Keywords: Dendrimers; 1,8-Naphthalimides; Fluorescence

1. Introduction

Dendrimers are well-defined three-dimensional macromolecules. In recent years, they have been receiving increased attention mainly because of their symmetry, high degree of branching and high density of the terminal functional groups, which can participate in different reactions [1–3]. Bonding a dye to the dendrimer structure gives the compounds new properties and new areas of applications [4,5]. Novel well-defined dendrimers with photoactive units at the core or at the periphery of the dendritic molecules have been reported and their photophysical properties investigated [6–17]. The fluorophore-labelled dendrimers [6–13] are among the most interesting in this aspect. Some dendrimers modified with cationically substituted 1,4,5,8-naphthalenediimide chromophoric groups have been investigated as electro-conductive materials [10–12]. Blue light emitting electroluminescent devices have been obtained using dendrimers modified with coumarin [13]. Dendrimeric liquid crystals [18–21] or dendrimer/liquid crystal mixtures [22,23] have been investigated with regard to their application in liquid crystalline displays. Their capacities as polymeric control drugs have also been tested [24]. The applications of 1,8-naphthalimides possessing bright colour, good photostability and physiological activities have also been widely studied. They are used in solar energy collectors [25], as laser active media [26,27], as potential photosensitive biologically active units [28], and in medicine [29]. Recently, they have been examined in liquid crystal systems for utilization in electro-optical devices [30–32]. The polyamidoamine (PAMAM) is a novel class of industrial dendrimers possessing a definite molecular composition and construction [1–3]. PAMAM dendrimers can also serve as macromolecular models for studying the influence of geometry on the transport across biological barriers [24]. Some 1,8-naphthalimide derivatives, which exhibit good fluorescence 'off–on switching' in the presence of protons or transition metal cations can perform as fluorescent sensors of photo-induced electron transfer (PET) [33–37]. They find applications in cell or molecular biological technology as well.

In this paper, we report on the synthesis of some

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PAMAM derivatives of a generation 0 bearing 1,8-naphthalimide fluorescent units in their peripheries. Belonging to the lowest generation, these novel compounds will be referred to as branched macromolecules (BM). Their photophysical characteristics have been investigated in organic solvents with different polarity. To the best of our knowledge, this is the first photoactive PAMAM containing 1,8-naphthalimide units with PET effect to be able to act as PET sensors for protons and transition metal cations.

2. Experimental part

2.1. Materials

PAMAM dendrimer of a generation 0 as a 20 wt% methanolic solution, 1,8-naphthalic anhydride and 4-nitro-1,8-naphthalic anhydride were used as obtained from Aldrich. The $Cu(NO_3)_2{\cdot}3(H_2O)$ was used as a cell for Cu^{2+} ions. *N,N*-dimethylformamide and methanol (MERCK) were used for the synthesis.

2.2. Synthesis

2.2.1. Synthesis of 1,8-naphthalimide-labelled PAMAM (BM1)

PAMAM methanolic solution (0.01 mol) and 0.04 mol 1,8-naphthalic anhydride were dissolved in 50 ml methanol. The solution was refluxed and the reaction was monitored by using TLC. After 5 h, the liquid was poured into 200 ml water and the resulting precipitate was filtered and dried in vacuum. Yield: 93%. FT-IR (KBr) cm^{-1}: 3373, 1699, 1656, 1625, 1588, 1384, 1236, 778. ^{1}H NMR (DMSO-d_6) δ (ppm) = 2.00 (d. 8H, CH_2–CO), 2.08 (d. 8H, CH_2–N–CH_2) 2.24 (d. 4H, N–CH_2CH_2–N), 3.17 (d. 8H, N–CH_2CH_2–NH), 4.07 (d. 8H, HN–CH_2), 7.74 (t, 4H, Ar–H), 8.31 (m, 20H, Ar–H), $C_{70}H_{64}O_{12}N_{10}$ (1236.2): Calcd C 67.96, H 5.17, N 11.32; Found C 67.21, H 5.03, N 11.23.

2.2.2. Synthesis of 4-nitro-1,8-naphthalimide-labelled PAMAM (BM2)

PAMAM methanolic solution (0.01 mol) and 0.04 mol of 4-nitro-1,8-naphthalic anhydride were dissolved in 50 ml of methanol. The solution was refluxed and the reaction was monitored by TLC. After 5 h, the liquor was poured into 200 ml of water and the resulting precipitate was filtered and dried in vacuum. Yield: 93%. FT-IR (KBr) cm^{-1}: 3387, 1787, 1664, 1625, 1528, 1343, 1233, 786. ^{1}H NMR (DMSO-d_6) δ (ppm) = 1.99 (d. 8H, CH_2–CO), 2.25 (d. 8H, CH_2–N–CH_2), 2.44 (d. 4H, N–CH_2CH_2–N), 3.16 (d. 8H, N–CH_2CH_2–NH), 4.06 (d. 8H, HN–CH_2), 7.94 (t, 4H, Ar–H), 8.40 (d, 4H, Ar–H), 8.46 (t, 8H, Ar–H), 8.55 (d, 4H, Ar–H). $C_{70}H_{60}O_{20}N_{14}$ (1360.2): Calcd C 61.76, H 4.41, N 14.41; Found C 60.99, H 4.29, N 14.15.

2.2.3. Synthesis of 4-piperidino-1,8-naphthalimide-labelled PAMAM (BM3)

4-Nitro-1,8-naphthalimide-labelled PAMAM BM2 (0.005 mol) was reacted with 0.04 mol of piperidine in 50 ml of *N,N*-dimethylformamide for 24 h at room temperature. After that 500 ml of water were added to the solution, the precipitate was filtered off, washed with water, then dried in vacuum at 40 °C. Yield: 87%. FT-IR (KBr) cm^{-1}: 3372, 1691, 1651, 1613, 1587, 1385, 1351, 1235, 783. ^{1}H NMR ($CDCl_3$) δ (ppm) = 1.75 (d. 40H, –CH_2–, aliph.), 2.38 (d. 8H, CH_2–CO), 2.75 (d. 8H, CH_2–N–CH_2), 3.14 (d. 4H, N–CH_2CH_2–N), 3.50 (d. 8H, N–CH_2CH_2–NH), 4.06 (d. 8H, HN–CH_2), 6.90 (t, 4H, Ar–H), 7.35 (d, 4H, Ar–H), 8.10 (m, 12H, Ar–H). $C_{90}H_{100}O_{12}N_{14}$ (1512.3): Calcd C 71.42, H 6.61, N 12.96; Found C 70.87, H 6.50, N 12.76.

2.2.4. Synthesis of 4-hexylamino-1,8-naphthalimide-labelled PAMAM (BM4)

The synthesis was run using the same procedure as BM3 using *n*-hexylamine. Yield: 76%. FT-IR (KBr) cm^{-1}: 3360, 1663, 1635, 1582, 1461, 1397, 1248, 775. ^{1}H NMR (DMSO-d_6) δ (ppm) = 0.85 (t. 12H, CH_3), 1.17 (m. 32H, –CH_2–, aliph.), 1.38 (q. 8H, $HNCH_2$–, aliph.), 1.50 (d. 8H, CH_2–CO), 1.65 (d. 8H, CH_2–N–CH_2), 3.01 (d. 4H, N–CH_2CH_2–N), 3.50 (d. 8H, N–CH_2CH_2–NH), 4.02 (d. 8H, HN–CH_2), 7.02 (t, 4H, Ar–H), 7.50 (d, 4H, Ar–H), 8.20 (m, 12H, Ar–H). $C_{94}H_{116}O_{12}N_{14}$ (1576.3): Calcd C 71.57, H 7.36, N 12.43; Found C 70.98, H 7.01, N 12.21.

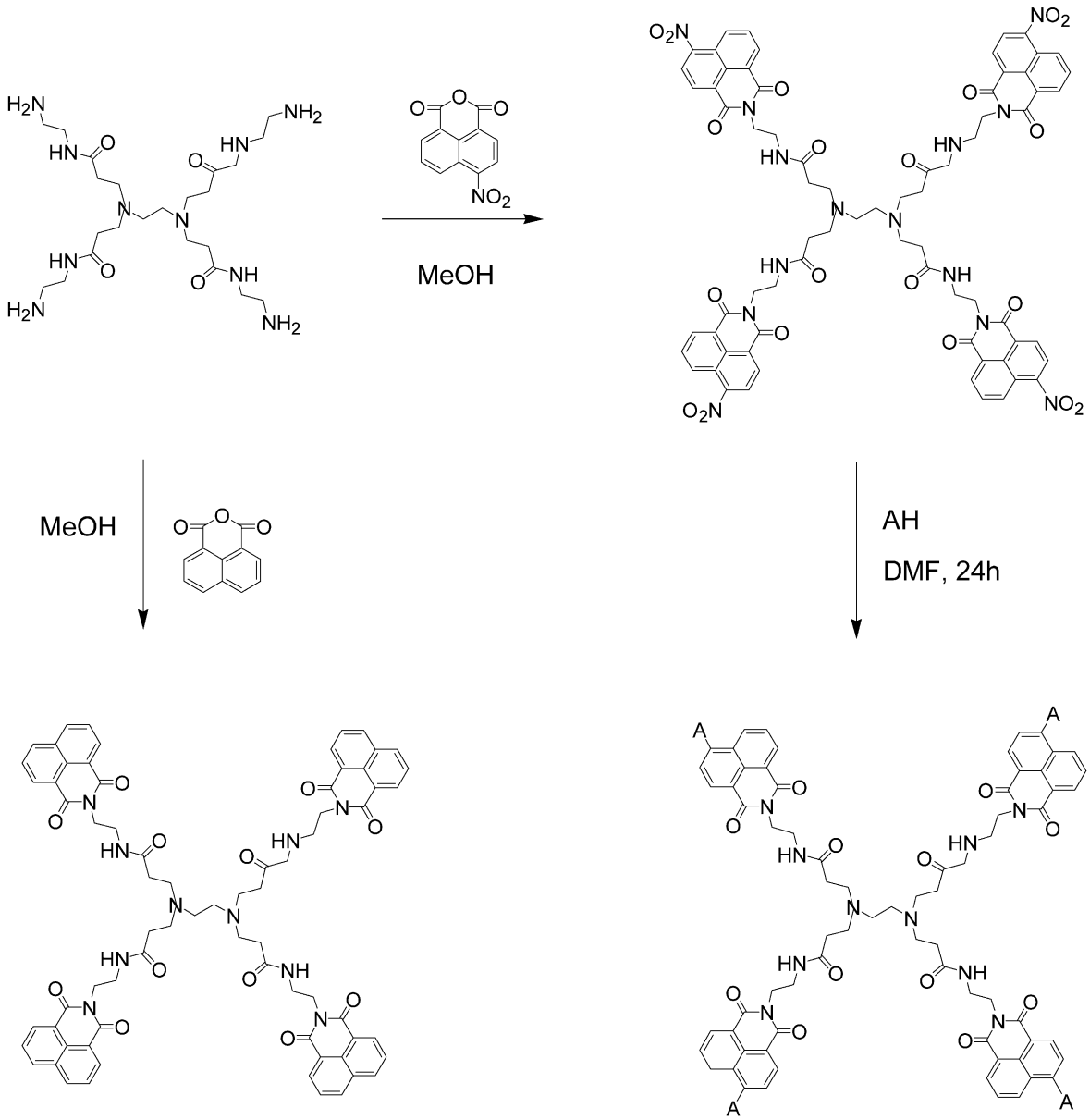
2.2.5. Synthesis of 4-4-N,N-dimethylaminoethylene-1,8-naphthalimide-labelled PAMAM (BM5)

The synthesis was run using the same procedure as BM3 using *N,N*-dimethylethyleneamine. Yield: 68%. FT-IR (KBr) cm^{-1}: 3394, 1681, 1637, 1581, 1457, 1427,1394, 1363, 775. ^{1}H NMR ($CDCl_3$) δ (ppm) = 2.32 (s. 24H, CH_3), 1.17 (m. 32H, –CH_2–, aliph.), 1.38 (q. 8H, $HNCH_2$–, aliph.), 2.40 (d. 8H, CH_2–CO), 2.61 (d. 8H, CH_2–N–CH_2), 2.70 (d. –CH_2N, aliph.), 3.14 (d. 4H, N–CH_2CH_2–N), 3.32 (s. $HNCH_2$–, aliph.) 3.50 (d. 8H, N–CH_2CH_2–NH), 4.05 (d. 8H, HN–CH_2), 7.28 (t, 4H, Ar–H), 7.55 (d, 4H, Ar–H), 8.10 (m, 12H, Ar–H). $C_{86}H_{104}O_{12}N_{18}$ (1580.3): Calcd C 65.31, H 6.58, N 15.94; Found C 64.89, H 6.43, N 15.76.

All the novel BMs 1–5 synthesized were purified by twofold recrystallisation in toluene.

2.3. Analysis

The UV–vis spectrophotometric investigations of the BMs 1–5 in dichloromethane and acetonitrile were performed on a Hewlett–Packard 845X spectrophotometer. Fluorescence spectra (dichloromethane and acetonitrile) were taken on a PTi spectrophotometer. The absorption and fluorescence spectra were recorded using 10^{-6} mol l^{-1} solutions of all the BMs. The quantum yield of fluorescence was determined by comparing the areas underneath the fluorescence spectra of the BMs and quinine sulphate as the



A = H (BM1), NO_2 (BM2), piperidino (BM3), $NH(CH_2)_5CH_3$ (BM4) and $NHCH_2CH_2N(CH_3)_2$ (BM5).

Scheme 1. Synthesis of new 1,8-naphthalimide-labelled PAMAM.

standard. IR spectra were measured on a Bruker IFS-113v spectrometer, using the KBr pellet technique at a 4 cm^{-1} resolution. The ^{1}H-NMR spectra of the BMs were recorded on a Varian INOVA 400 NMR spectrometer at 400 MHz. DMSO-d_6, $CDCl_3$ and tetramethylsilane was used as a solvent and as an internal standard, respectively. Thin layer chromatography (TLC) analysis was performed on silica gel (Fluka F_{60} 254 20 × 20; 0.2 mm) using the solvent system *n*-heptane/acetone (2:1) as eluent.

3. Results and discussion

3.1. Synthesis of branched macromolecules 1–5

Scheme 1 presents the route to the synthesis of the new 1,8-naphthalimide-labelled PAMAMs.

New 1,8-naphthalimide-labelled BMs were prepared from generation 0 PAMAM, which possesses four primary amine groups. 1,8-naphthalic anhydride reacted with

Table 1
Photophysical characteristics of BMs 1–5 in dichloromethane

	BM1	BM2	BM3	BM4	BM5
λ_A (nm)	335	351	417	434	433
log ϵ	4.74	4.68	4.48	4.21	4.50
λ_F (nm)	472	–	525	518	516
$\nu_A - \nu_F$ (cm^{-1})	8664	–	4933	3736	3715
Φ_F	0.017	–	0.151	0.171	0.080

PAMAM in methanol solution, giving new compound containing 1,8-naphthalimide units (BM1). The same procedure was used to obtain a 4-nitro-1,8-naphthalimide-labelled derivative (BM2). The BM1 and BM2 were obtained as a white solid by filtration. The reaction and purity of BM1 and BM2 were monitored by TLC. 1,8-naphthalimide-labelled yellow–green fluorescent BMs 3–5 were obtained in good yields by nucleophilic substitution of the nitro group of 4-nitro-1,8-naphthalimide-labelled PAMAM (BM2) in *N*,*N*-dimethylformamide solution at ambient temperature for 24 h. In this case, the electron-accepting carbonyl group of the naphthalimide molecule favours the nucleophilic substitution reactions of the nitro group with aliphatic amines [32]. The final products BMs 3–5 were obtained after precipitation with water and filtration of the solid precipitate which was subjected to double recrystallisation from toluene.

3.2. Photophysical and PET properties

The BMs 1–5 under study are soluble in organic solvents, but not in water. Their photophysical characteristics were recorded in two organic solvents with different polarity: dichloromethane ($\epsilon = 8.93$) and acetonitrile ($\epsilon = 37.5$). Tables 1 and 2 present the absorption and fluorescence spectral characteristics of the BMs 1–5 in solution: the absorption (λ_A) and fluorescence (λ_F) maxima, the extinction coefficient (log ϵ), Stokes shift ($\nu_A - \nu_F$), and quantum yield of fluorescence (Φ_F).

In dichloromethane, the UV absorption maxima of BM1 and BM2 are at 335 and 351 nm, respectively, while in acetonitrile solutions, the maxima appear at 332 and 349 nm, respectively. Introduction of an electron-donating amino group at C-4 position into the naphthalimide structure in the case of BMs 3–5 induces a significant bathochromic shift. Then, the fluorophores absorption maxima are in the 417 ÷ 434 nm region in dichloromethane, and in acetonitrile solutions, in the 412 ÷ 437 nm region. In this case, the BMs 3–5 are yellow–green in colour. The compounds BM4 and BM5, having a secondary alkyl amino groups as substituents of the naphthalimide, give maxima bathochromically shifted if compared with the absorption maximum of BM3 comprising a tertiary amino substituent at position C-4. In this case, the extinction coefficients are significantly high ($\varepsilon > 16\,500$ mol l^{-1} cm^{-1}), indicating that the long-wavelength band of the absorption spectra is a band of charge transfer (CT), reflecting on the (π,π^*) character of the $S_0 \rightarrow S_1$ transition of BMs 3–5. The shift in the CT bands for BMs 3–5 in the solvents is caused by the solvatochromic effect of the solvents used.

Table 2
Photophysical characteristics of BMs 1–5 in acetonitrile

	BM1	BM2	BM3	BM4	BM5
λ_A (nm)	332	349	412	437	431
log ϵ	4.69	4.54	4.42	4.22	4.48
λ_F (nm)	471	–	539	530	529
$\nu_A - \nu_F$ (cm^{-1})	8889	–	5719	4016	4292
Φ_F	0.016	–	0.200	0.112	0.011

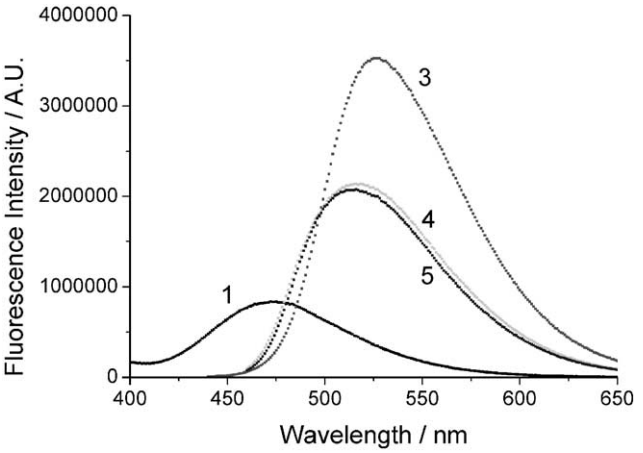


Fig. 1. Fluorescence emission spectra of BMs 1, 3–5 in solution of dichloromethane ($c = 1 \times 10^{-6}$ M).

Fig. 1 presents the fluorescence spectra of BMs 1, 3–5 in dichloromethane. In the case of BM1, a blue fluorescence with a well-pronounced maximum at 472 nm has been obtained. There are no data on the fluorescence of BM2 as the electron accepting nitro groups in this compound quench the fluorescence. In these solutions, BMs 3–5 have a yellow–green fluorescence, with maxima in the 516–525 nm region.

The Stokes shift is an important parameter for the fluorescent compounds. This parameter indicates the difference in the properties and structures of the compounds between the ground state S_0 and the first exited state S_1. The Stokes shift has been estimated according to Eq. (1):

$$(\nu_A - \nu_F) = (1/\lambda_A) - (1/\lambda_F) \times 10^{-7} \quad (1)$$

The Stokes shift calculated is 4292–8889 cm^{-1} in acetonitrile and 3715–8664 cm^{-1} in a dichloromethane solution. In both the cases, the Stokes shift is larger in more polar acetonitrile solution (Tables 1 and 2). Much larger Stokes shifts, 8664 and 8889 cm^{-1}, are observed in the case of BM1. This indicates conformational changes in the chromophoric naphthalimide systems. In the case of BMs

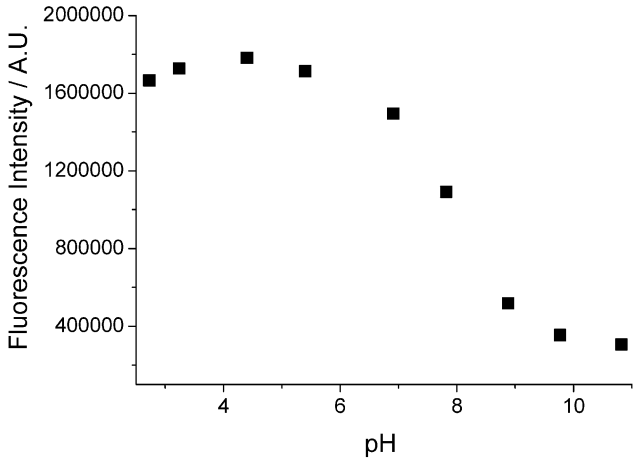


Fig. 2. pH dependence of fluorescence intensity of BM5 in MeOH/H_2O (1:4, v/v). The pH values are in the order of decreasing intensity, 2.73, 3.34, 5.54, 6.91, 7.82, 8.88, 9.77, and 10.82. The excitation wavelength is 432 nm. The concentration of BM5 is 1×10^{-6} M.

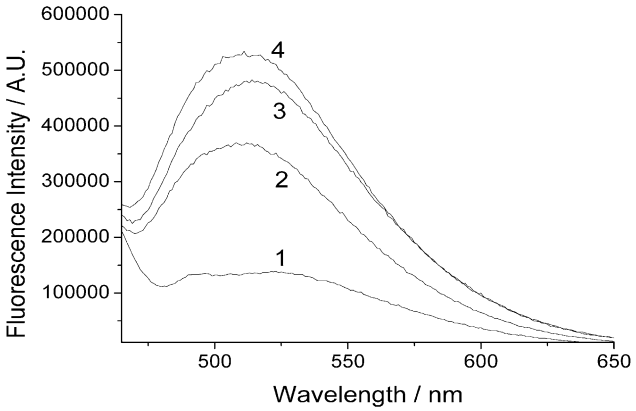


Fig. 3. Fluorescence spectra of BM5 in acetonitrile at various concentrations of Cu^{2+} cations. The concentrations of Cu^{2+} cations are in the order of increasing intensity, (1) 0, (2) 5×10^{-5} M, (3) 5×10^{-4} M, (4) 9.6×10^{-4} M. The concentration of BM5 is 1×10^{-6} M.

3–5, the amino substituents probably are able to stabilize the rotations of the molecule and the conformational changes are small.

The ability of the molecules to emit absorbed light energy has been characterized quantitatively by the fluorescence quantum yields (Φ_F). It has been determined on the basis of the absorption and fluorescence spectra in dichloromethane and acetonitrile at a concentration of 10^{-6} mol l^{-1} with quinine bisulphate as the standard ($\Phi_F = 0.55$) [38]. The data presented in Tables 1 and 2 show that the fluorescence quantum yield (Φ_F) is greater in the case of BM3. A smaller Φ_F is observed for BM1. The fluorescence quantum yield of BM4 having an *n*-hexylamino group at C-4 position ($\Phi_F = 0.171$ in acetonitrile) has been observed. Compared with the monomeric 4-hexylamino-1,8-naphthalimides [39], the present values obtained are very low. Similarly, the fluorescence quantum yield ($\Phi_F = 0.20$ in acetonitrile) of BM3 having an *n*-hexylamino group at C-4 position is also lower than that of similar monomeric *N*-alkyl-1,8-naphthalimide (0.30 in acetonitrile) [40]. This phenomenon might be caused by the possible PET from the amino groups of the central part of PAMAM to the core of naphthalimide units [41]. A much lower Φ_F (0.011 in acetonitrile) has also been determined in the case of BM5, having chromophore 4-*N*,*N*-dimethylaminoethylamino-1,8-naphthalimide as the residue. The similar monomeric 4-*N*,*N*-dimethylaminoethylamino-*N*-alkyl-1,8-naphthalimide has a little bit higher Φ_F (0.018 in acetonitrile) [42] as well, which has very well-pronounced PET properties in the presence of protons or transition metal ions [37]. In BM5, the naphthalimide core units are subjected to a PET from the atoms comprised in the central amino groups of PAMAM and to another one from the terminal amino groups of dialkylamino moieties. Of course, the latter PET is a major factor.

Fig. 2 presents the pH dependence on fluorescence intensity of BM5. As is seen the fluorescence intensity depends strongly on pH values in the 2.73–10.82 region. The results show that the protonation of the amino groups of the central PAMAM part and that of the terminal dimethylamino moiety of the naphthalimide increase the florescence intensity. A hypsochromic shift of the fluorescence maxima ($\Delta\lambda = 10$ nm) in acid medium has been observed. It is an indication of an interaction between H^+ and the branched molecules. The fluorescence enhancement is 5.8, which testifies a strong PET fluorescence quenching.

Fig. 3 shows the effect of the Cu^{2+} ions upon the fluorescence spectrum of BM5 in acetonitrile solution. With the addition of Cu^{2+} cations into the solution up to a concentration of 1.2×10^{-4} M the fluorescence emission increases. In this case, the fluorescence enhancement is 4.3 times larger. But with further rise in the concentration the fluorescence intensity decreases being quenched by the high concentration of Cu^{2+} cations. The presence of Cu^{2+} cations induces a hypsochromic shift in the fluorescence maxima ($\Delta\lambda_F = 12$ nm), and also indicates the interaction between the BM and metal ions in the exited state.

Similar results have been achieved when studying single 4-*N*,*N*-dimethylethylamino-*N*-allye-1,8-naphthalimide [43]. The results reveal that the protonation of the terminal dialkylamino group of the naphthalimide molecule increases the florescence intensity. The fluorescence emission increases too after the addition of the Cu^{2+} ions into the dye solution up to a concentration of 1.25×10^{-5} M, which was corresponding to the formation of intramolecular bisdental chelation with Cu^{2+} cations. The present study is in agreement with these results and confirms that the naphthalimide structure of the compounds is a determining factor in the PET process proceeding in the BMs discussed.

4. Conclusion

The first synthesis of a new 1,8-naphthalimide-labelled PAMAM derivatives has been described. Their

photophysical characteristics have been determined in two organic solvents of different polarity. The derivatives comprising a hydrogen atom or a nitro group as a substituent at C-4 position in the naphthalimide structure are colourless and absorb in the UV region, whereas those having an amino group for a substituent absorb in the visible region and emit yellow–green fluorescence. The synthesis of the green fluorescent 1,8-naphthalimide-labelled PAMAM BM5 with designed properties of a fluorescent sensor of PET has been suggested for the first time. It has been shown that the fluorescence emission of this novel compound increases strongly in the presence of protons or Cu^{2+} cations.

Acknowledgments

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