

New fluorescent PAMAM dendron with sensor and microbiological activity

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A new dendron containing Eosin Y as fluorescence unit has been synthesized and characterized. The basic photo-physical characteristics of dendron have been investigated in organic solvents with different polarity and the results have been compared to the modified Eosin Y. In *N,N*-dimethylformamide solution, the newly synthesized dendron quench its fluorescence intensity in the presence of metal cations (Li^+ , Na^+ , K^+ , Ag^+ , Co^{2+} , Zn^{2+} , Mn^{2+} , Ni^{2+} , Cu^{2+} , Fe^{3+} and Cr^{3+}) depending from the nature of metal cations. It has also been observed that the fluorescence intensity is not affected from pH of the medium. Antimicrobial activity of the new dendron was tested in meat-peptone broth towards some model bacteria and yeasts.

Keywords: Eosin Y, dendron, PAMAM, sensor, environment pollutants, antimicrobial activity.

INTRODUCTION

The monitoring and rapid control of the environment pollutants is at the core of the development of new analytical systems for their detection. Especially suitable for these purposes are fluorescence optical sensors responsive to light irradiation in the presence of certain analytes with a change of their fluorescence emission. Therefore, in recent years, research has been focused on finding the possibility to increase the sensitivity, selectivity and appropriate working range of new sensor systems, depending on their application areas. An innovative area is the use of dendrimer or dendritic macromolecules, which are a new form of organization of polymeric materials [1]. For sensing applications dendrimers must be functionalized with fluorescent dyes because dendrimer molecules do not exhibit intrinsic photophysical properties. As fluorescent fluorophores for dendrimer and dendron modification are used 1,8-naphthlimide, xanthene dyes, dansyl, acridine, naphthalene, etc. [2–7].

In our laboratory we perform systematic investigations on the synthesis and photophysical properties of new luminescent polyamidoamine (PAMAM) and polypropyleneamine (PPA) dendrimers modi-

fied with 1,8-naphthamide units in their periphery [7]. Several fluorescent tripods based of 1,8-naphthamide with sensor properties have also been described [8–10]. Xanthene dyes, such as eosin Y, are a very interesting and special class of environmentally sensitive fluorophores which participate as a signal fragment in the design of different sensor systems [11]. They have low toxicity *in vivo*, and moderately high water solubility. They exhibit different tautomeric structures with different photolytic forms, either proton “on” or “off” depending on pH [12].

In this paper we describe the synthesis and characterization of a new PAMAM dendron from second generation. Its basic photophysical characteristics have been investigated in organic solvents of different polarity. The behaviour of fluorescent intensity in media with different pH and the ability to detect metal ions have been investigated. *In vitro* antimicrobial activity against Gram-positive and Gram-negative bacteria and yeasts has also been tested.

EXPERIMENTAL

Materials and methods

The synthesis of acylated eosin Y (E) with chloroacetyl chloride has been described recently (Scheme 1) [13]. Dendron D has been synthesized

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by the procedure described by Ghosh et. al. [14]. Absorption spectra were performed using “Thermo Spectronic Unicam UV 500” spectrophotometer. The fluorescence spectra were taken on a “Cary Eclipse” spectrophotometer. Organic solvents were of spectroscopic grade and used without special treatment. The effect of the metal ions on fluorescence intensity was measured by adding a few μl of stock solution ($c = 10^{-3} \text{ mol l}^{-1}$) of the metal cations to a known volume of the ligand solution (3 ml). The addition was limited to 0.08 ml, so that dilution remained insignificant [15]. NaNO_3 , KNO_3 , LiNO_3 , AgNO_3 , $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$, $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{Mn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{Zn}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, and $\text{Cr}(\text{NO}_3)_3$, $\text{Fe}(\text{NO}_3)_3$ salts were the metal cation sources, and were used as obtained from Sigma-Aldrich. IR spectra were recorded on an Infrared Fourier transform spectrometer (IRAffinity-1 Shimadzu) with the diffuse-reflectance attachment (MIRacle Attenuated Total Reflectance Attachment) at a 2 cm^{-1} resolution. ^1H (600.13 MHz) and ^{13}C (150.92 MHz) spectra were acquired on an AVANCE AV600 II+NMR spectrometer. The measurements were carried out in a DMSO-d_6 solution at ambient temperature. The chemical shifts were referenced to a tetramethylsilane (TMS) standard. Electrospray mass spectroscopic measurements were carried out using a Hewlett–Packard Series 1100 MSD.

Synthesis of dendron ED

A solution 0.709 g (1mmol) of compound **E** and 1.24g, (1mmol) of **D** in 50 ml acetone was refluxed in the presence of 0.1500, (1.1 mmol) K_2CO_3 for 8 hours. The process was controlled by thin-layer chromatography and the final product was filtered off with very high yield and purity after pouring the liquor into 500 ml of water. The resulting precipitate was washed with water, and then dried in vacuum at 40°C . Yield: 1.36g, 85.0%.

FT-IR (KBr) cm^{-1} : 1736, 1652, 1619, 1557, 1507, 1455, 1346, 1230, 1085, 1057, 973, 879, 762, 707, 645; $^1\text{H-NMR}$ (DMSO-d_6 , 600 MHz, ppm): 9.06 (s, 1H, COOH), 8.42 (d, 1 H, $j = 7.8 \text{ Hz}$, H-Ar), 8.08 (t, 2H, $j = 5.6 \text{ Hz}$, NHCO), 7.90 (d, 1 H, $j = 7.4 \text{ Hz}$ H-Ar), 7.64 (d, 1 H, $j = 7.2 \text{ Hz}$, H-Ar), 7.38 (d, 1 H, $j = 7.8 \text{ Hz}$, H-Ar), 6.90 (t, 1 H, $j = 7.8 \text{ Hz}$, H-Ar), 4.42 (t, 2H, $j = 6.4 \text{ Hz}$, $-\text{OCH}_2\text{-CO}$), 3.80 (m, 18H, N- CH_2), 3.61 3.62 (s, 12H, OCH_3), 3.44 (br. 2H, OCH_2), 3.22 (q, 4H, $\text{NCH}_2\text{CH}_2\text{CONH}$), 2.91 (br, 4H, $\text{NCH}_2\text{CH}_2\text{NH}$), 2.64 (t, 8H, $j = 6.5 \text{ Hz}$, $\text{NCH}_2\text{CH}_2\text{COOCH}_3$) 2.33 (t, 8H, $j = 6.5 \text{ Hz}$, $\text{NCH}_2\text{CH}_2\text{COOCH}_3$). API-ES-MS calc. for (1320.8) found 1321.7 (M+H) $^+$; Elemental analysis: $\text{C}_{50}\text{H}_{59}\text{N}_5\text{O}_{17}\text{Br}_4$ (1320.8): C 45.42% (calc. 45.58%); H 4.46% (calc. 4.39%); N 5.30% (calc. 5.41%)

Antimicrobial assay

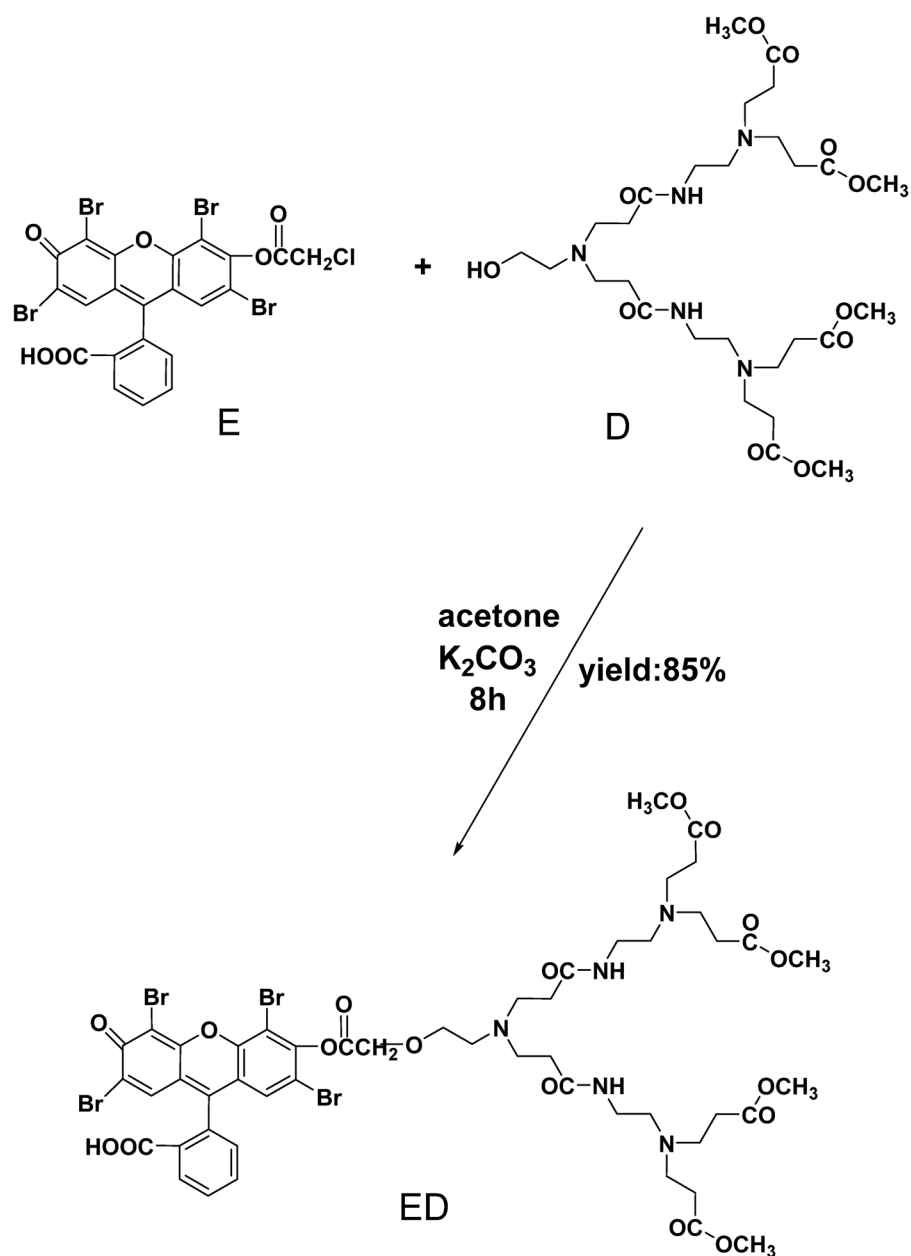
The antimicrobial activity of the newly synthesized dendron ED was evaluated in meat-peptone broth towards Gram-positive bacterium *Bacillus subtilis*, Gram-negative bacterium *Pseudomonas aeruginosa* and the yeasts *Candida lipolytica*. Aliquots of sample solution in DMSO (0.1%) were added in test tubes with sterile meat-peptone broth achieving final concentration $50 \mu\text{g/ml}$ and then inoculated with 1% of each overnight indicator culture. Test tubes without added compound were also prepared for each strain. After incubation of the strains at 26°C for 24h under shaking, the microbial growth was determined by measuring the optical density at 600 nm (OD_{600}). Three independent experiments were carried out and averages are reported.

RESULTS AND DISCUSSION

Scheme 1 presents the synthetic rout of fluorescent dendron ED. Initial compound (**E**) was synthesised by the reaction of eosin Y with chloroacetyl chloride following the method described recently [13]. This compound has been used as fluorescent units for the synthesis of fluorescent PAMAM dendron by the reaction with dendron **D**. The final solid product (**ED**) was obtained in high yield, filtrated, washed with water and dried under vacuum.

The basic spectral characteristics of compound **E** and Dendron **D** have been investigated in organic solvents with different polarity and the results have been summarised in Table 1 and Table 2: the absorption (λ_A) and fluorescence (λ_F) maxima, the extinction coefficient (ϵ), Stokes shift ($\nu_A - \nu_F$), and quantum yield of fluorescence (Φ_F).

Compound **E** has absorption maxima in the region $\lambda_A = 528\text{--}540 \text{ nm}$ and the respective fluorescence maxima are at $\lambda_F = 547\text{--}560 \text{ nm}$. These results suggest that the polarity of the organic solvents influence the position of the absorption and fluorescence maxima. At the organic solvents containing hydroxyl groups, both types of maxima are hypochromically displaced, with this effect being most pronounced for methanol. This is due to the possibility of the formation of hydrogen bonds. The similar results have been obtained for the dendron ED. That means the bonding to the dendron chain do not affect the chromophoric system of the monomeric compound **E**. The normalized absorption and fluorescence spectra of dendron ED in n-butanol solution are plotted in Fig. 1. As seen the absorption and fluorescence spectra have bands with a single maximum, without vibrational structure. The fluorescence curve is an approximately equal mirror



Scheme 1. Synthesis of Dendron ED.

Table 1. Photophysical characteristics of compound E in organic solvents

	λ_A nm	ϵ $l \text{ mol}^{-1} \text{ cm}^{-1}$	λ_F nm	$\nu_A - \nu_F$ cm^{-1}	$\lambda\Phi_F$	f
Acetonitrile	539	61300	557	600	0.64	0.345
<i>N,N</i> -dimethylformamide	538	64200	556	602	0.60	0.349
<i>n</i> -Buthanol	530	68000	550	686	0.62	0.366
2-propanol	531	54720	549	617	0.58	0.249
Methanol	528	58970	547	657	0.54	0.289
Chloroform	537	55320	556	636	0.29	0.256
Dichlorometane	537	54300	560	764	0.26	0.241
Tetrahydrofuran	540	66700	559	629	0.28	0.371

Table 2. Photophysical characteristics of dendron **ED** in organic solvents

	λ_A nm	ϵ l mol ⁻¹ cm ⁻¹	λ_F nm	$\nu_A - \nu_F$ cm ⁻¹	Φ_F	f
Acetonitrile	538	114000	560	730	0.76	0.542
<i>N,N</i> -dimethylformamide	536	119000	556	671	0.66	0.539
n-Butanol	533	129000	559	872	0.64	0.599
2-propanol	534	130000	560	869	0.56	0.590
Methanol	526	114250	557	1058	0.49	0.534
Chloroform	537	113300	557	668	0.21	0.496
Dichlorometane	536	130600	557	703	0.20	0.558
Tetrahydrofuran	539	149385	560	695	0.24	0.603

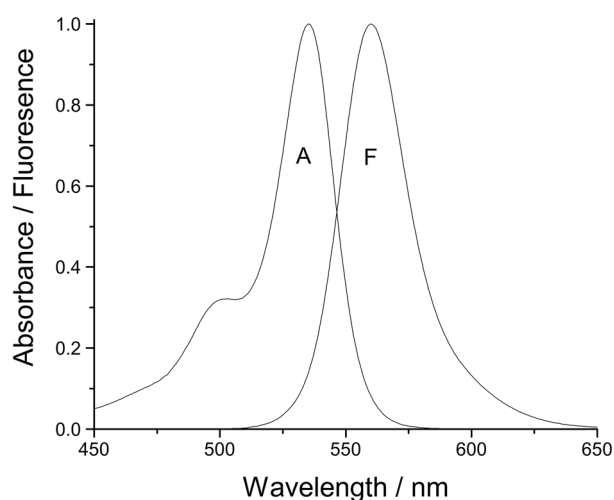
**Fig. 1.** Normalized absorption (A) and fluorescence (F) spectra of dendron ED in butanol solution ($c = 1 \times 10^{-6}$ mol l⁻¹).

image of the absorption curve what is indicative of the molecular structure of the dendrimer in excited state and prevailing fluorescence emission.

An important characteristic of the fluorescence compounds is the oscillator strength (f) which reveals the effective number of electrons whose transition from ground S_0 to excited S_1 state gives the absorption area in the spectrum. Values of the oscillator strength can be calculated using Equation (1):

$$f = 4.32 \times 10^{-9} \Delta\nu_{1/2} \epsilon_{\max} \quad (1),$$

where $\Delta\nu_{1/2}$ is the width of the absorption band (in cm⁻¹) at $1/2 \epsilon_{\max}$.

The values obtained for **E** are: $f = 0.249$ – 0.366 and for the dendron **ED** the values are higher $f = 0.496$ – 0.603 . The higher values correlate well with the hypochromic effect of the **ED** in the respective organic solvents.

The Stokes shift is a parameter, which indicates the difference in the properties and structure of the dyes between the ground state S_0 , and the first excited state S_1 and it has been estimated according to Equation (2):

$$(\nu_A - \nu_F) = (1/\lambda_A - 1/\lambda_F) \times 10^{-7} \quad (2).$$

The Stokes shift values for both compounds are very similar and do not imply any change in the chromophores systems in the excited state. The values are typical for this class of compounds.

The ability of compounds **E** and **ED** to emit absorbed light energy has been characterized by the fluorescence quantum yield Φ_F using Rhodamine 6G as a standard. It has been calculated on the basis of the absorption and fluorescence spectra in organic solvents using Equation (3).

$$\Phi_F = \Phi_{st} \frac{S_u A_{st} n_{Du}^2}{S_{st} A_u n_{Dst}^2} \quad (3)$$

where the Φ_F is the emission quantum yield of the sample, Φ_0 is the emission quantum yield of standard, A_{st} and A_u represent the absorbance of the standard and sample, respectively, while I_{st} and I_u are the integrals of the emission of the standard and sample respectively, and n_{Dst} and n_{Du} is the refractive index of the standard and sample.

As seen from the data in Table 1 and Table 2, the quantum yield depend strongly from the polarity of the solvents. Higher values have been obtained in polar solvents which are more than three times compared to the non polar solvents.

Influence of pH on the fluorescence intensity of E and ED

The influence of pH on the absorbance and fluorescence intensity of compounds **E** and **ED** have

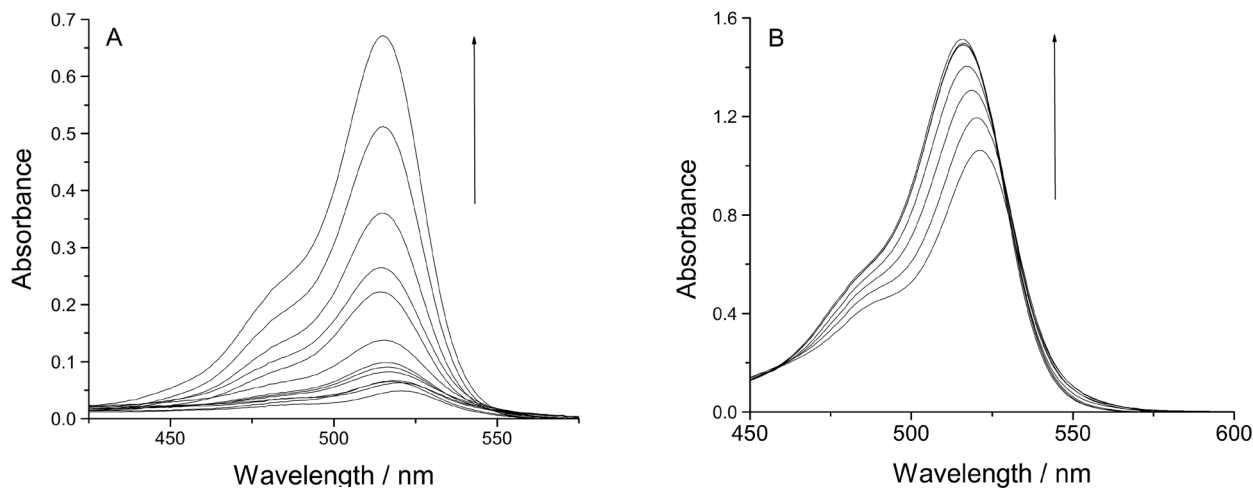


Fig. 2. Effect of pH in range from 3.5 to 12.0 on absorbance of compound **E** (A) and Dendron **ED** (B) in ethanol/water solution (1:4 v/v).

been investigated in ethanol/water (1:4 v/v) solution. In Fig. 2 are plotted the absorption spectra of both compounds in the pH range from 3.5 to 12.0. In the case of monomer compound **E** ($c = 1 \times 10^{-6} \text{ mol l}^{-1}$) the absorption maxima change its position from $\lambda_A = 522 \text{ nm}$ in acidic medium to $\lambda_A = 515 \text{ nm}$ (Fig. 2A). Also, a drastic increase in the molar absorbance of the compound was observed, with the molar extinction coefficient of $\epsilon = 49900 \text{ l mol}^{-1} \text{ cm}^{-1}$ in acidic medium increasing to $\epsilon = 67100 \text{ l mol}^{-1} \text{ cm}^{-1}$ in the alkaline medium. The same investigations were conducted for dendron **ED** and the results obtained are shown in Fig. 2B. It can be seen the effect of pH on absorption of dendron **ED** is less pronounced. In this case, a hypochromic displacement of the absorption maximum was observed by 10 nm, but the molar extinction coefficient increased significantly less.

Figure 3 presents the normalized fluorescence intensity of compounds **E** and **ED** depending on the pH of the medium. Figure 3 shows that both compounds have different behaviour. For compound **E**, the fluorescence intensity slightly increased in the interval $\text{pH} = 3.5\text{--}10.0$ after which in strong alkaline medium ($\text{pH} = 10\text{--}12$) the emitted fluorescence has increased significantly. In the case of dendron **ED**, the influence of pH of the medium is negligible and the fluorescence has almost the same intensity in the interval of $\text{pH} 3.5\text{--}12.0$.

Influence of metal cations on the photophysical properties of ED

Different metal ions (Li^+ , Na^+ , K^+ , Ag^+ , Co^{2+} , Zn^{2+} , Mn^{2+} , Ni^{2+} , Cu^{2+} , Fe^{3+} and Cr^{3+}) upon the fluorescent intensity of DMF solutions of **ED** has been

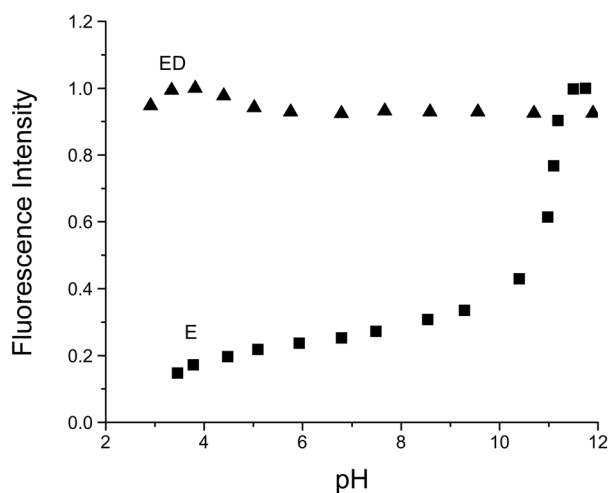


Fig. 3. Dependence of fluorescence intensity from pH of compounds **E** and **ED**.

studied with regards to its potential applications as a detector for metal ions. DMF has been chosen for the fluorescence experiments because it is a good solvent for dendron **ED**, metal salts used as well as for the formed metal complex.

In DMF solution the free of metal cations, dendron **ED** has a good fluorescence emission with quantum yields $\Phi_F = 0.66$. Fluorescence emission decreases after the addition of metal ions into the solution and the effect of the guest metal cations in the solution is signalled by fluorescence quenching factor (FQ) (Fig. 4). The fluorescence enhancement factor $FQ = F_0/F$ is determined from the ratio of initial fluorescence intensity (F_0 before metal ions addition) and minimum fluorescence intensity (F after addition of metal ions). As seen in Fig. 3, the

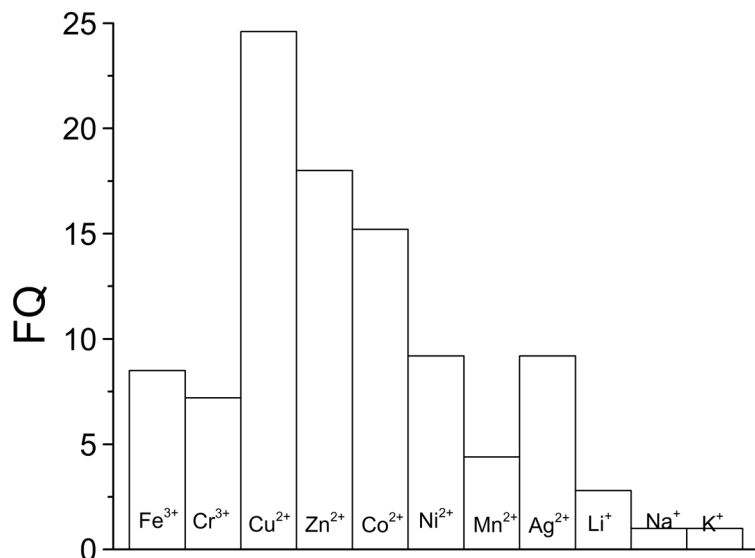


Fig. 4. Fluorescence quenching factor (*FQ*) of **ED** ($c = 1 \times 10^{-6} \text{ mol l}^{-1}$) in the presence of different metal cations ($c = 1 \times 10^{-6} \text{ mol l}^{-1}$) in DMF solution.

metal cations lead to a decrease in the fluorescence intensity which is different for each metal ion. The highest value is observed in the presences of Cu^{2+} ($\text{FQ}=24.6$). A good reduction of the fluorescent emission has been observed for Zn^{2+} and Co^{2+} . An average fluorescence quenching has been observed in the presence of Ag^+ , Ni^{2+} , Cr^{3+} and Fe^{3+} . Metal ions such as Li^+ and Mn^{2+} cause a slight reduction, while alkali metal ions as Na^+ and K^+ , have not effect on the fluorescence intensity of dendron **ED**. The rank of the response can be presented as follows: $\text{Cu}^{2+} > \text{Zn}^{2+} > \text{Co}^{2+} > \text{Ni}^{2+} \approx \text{Ag}^+ \approx \text{Fe}^{3+} \approx \text{Cr}^{3+} > \text{Mn}^{2+} > \text{Li}^+ > \text{Na}^+ \approx \text{K}^+$.

Most probably during the complex formation Cu^{2+} are the most competitive in the reaction with tertiary nitrogen atoms comprised dendron structure. That ensures a stability of the complex thus formed which is greater than that of complexes formed with the other metal ions studied.

In order to quantify the effect of Cu^{2+} on the fluorescence intensity we have performed detailed titration experiments. The typical change in the fluorescence intensity of **ED** induced by Cu^{2+} is plotted in Fig. 5 as an example. As seen, the addition of Cu^{2+} leads to a reduction in the fluorescence intensity. A pronounced increase in the fluorescence intensity

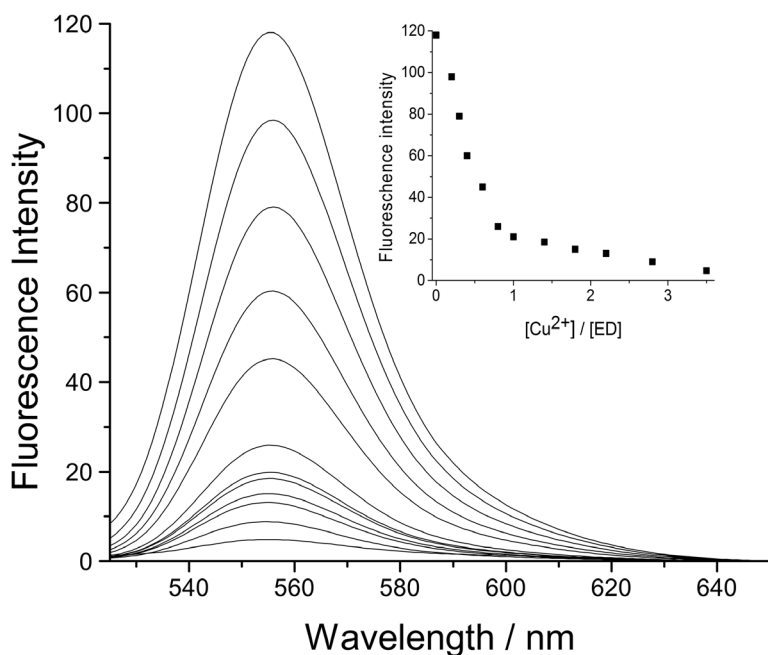


Fig. 5. Fluorescence spectra of **ED** ($c = 1 \times 10^{-6} \text{ mol l}^{-1}$) in DMF at various concentrations of Cu^{2+} cations. The concentrations of Cu^{2+} cations are in the order of increasing intensity from 0 to $4 \times 10^{-6} \text{ mol l}^{-1}$.

has been observed by increasing the concentration of Cu^{2+} cations up to $1.0 \times 10^{-6} \text{ mol l}^{-1}$ and above this concentration the change in the fluorescence intensity is negligible. The inset in Fig. 5 shows the plots of fluorescence intensity versus Cu^{2+} concentration which demonstrate that one metal ion coordinate with **ED**. From Fig. 5 also it is seen that during the titration with Cu^{2+} ions the position of the fluorescence maximum do not change its position in the presence of Cu^{2+} ions. This indicates that Cu^{2+} ions do not coordinates directly with the chromophore system of Eusin Y unites. The possible coordination of metal ions with dendron **ED** is with him aliphatic part as it is presented in Scheme 2.

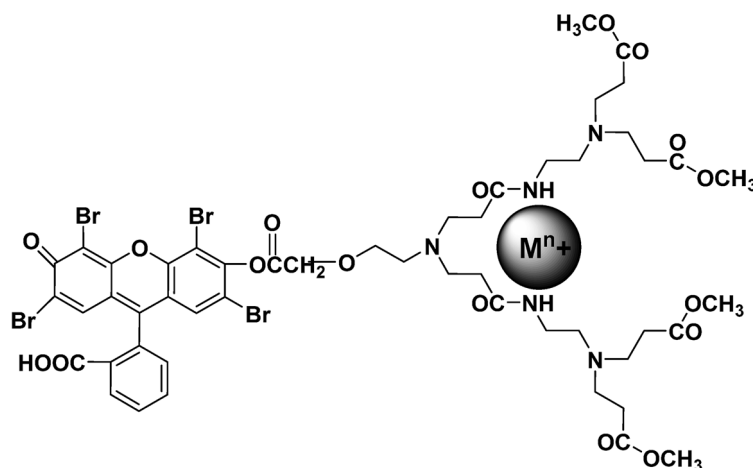
The fluorescence titration profile shows that a linear curve appears ($R = 0.982$) in $0 \div 8 \times 10^{-7} \text{ mol l}^{-1}$ concentration range of Cu^{2+} cations (Fig. 6). A

detection limit of $9.2 \times 10^{-8} \text{ mol l}^{-1}$ for Cu^{2+} has been determined [16]. Such a linear curve and a detection limit are sufficient enough to sense and determine Cu^{2+} ions.

In this case the formed metal complex probably destabilizes the planarity of the Eosin Y chromophore system. The non-radiative deactivation during the transition from S_1 to the S_0 prevails leads to decrease in the fluorescence quantum yield (Fig. 7). The lowest Φ_F has been observed in the presence of Cu^{2+} cations ($\Phi_F = 0.02$).

Antimicrobial activity of **ED**

The results for antimicrobial activity showed that the new dendron **ED** in concentration $50 \mu\text{g/ml}$ inhibited the growth of the used test cultures (Fig. 8).



Scheme 2. Proposed mechanism of complexation.

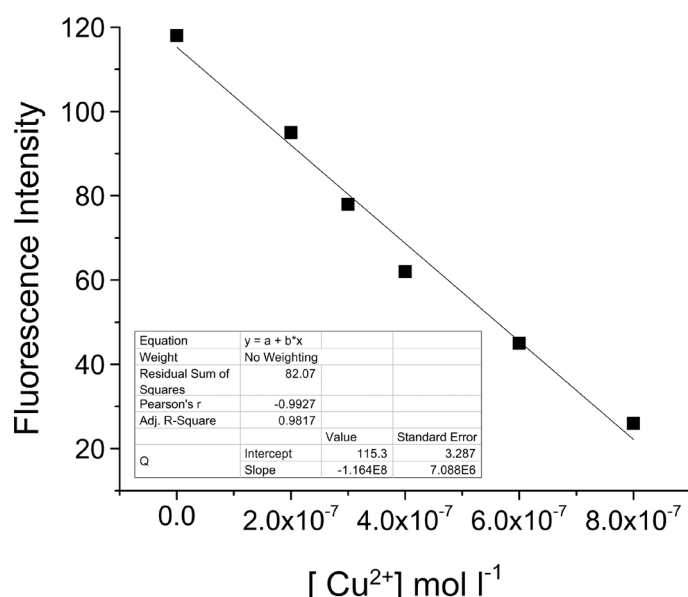


Fig. 6. Dependence of fluorescence intensity of dendron **ED** ($c = 1 \times 10^{-6} \text{ mol l}^{-1}$) in the presence of different amount of Cu^{2+} .

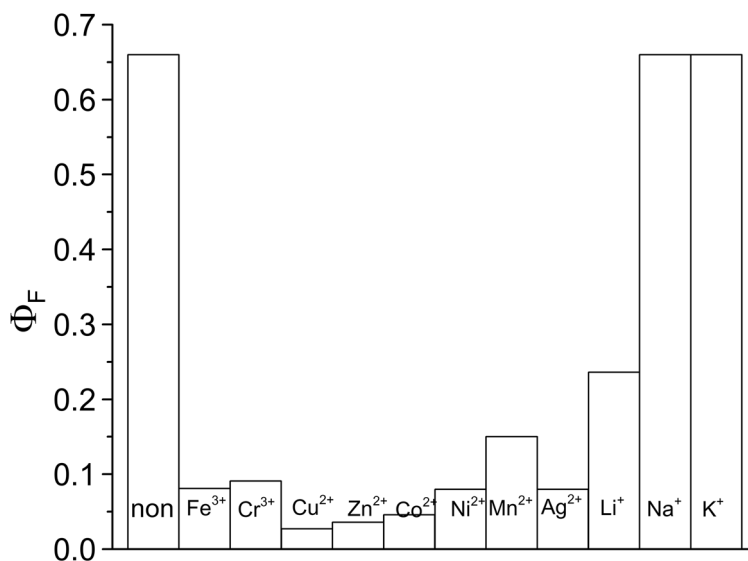


Fig. 7. Quantum yield of dendron ED ($c = 1 \times 10^{-6} \text{ mol l}^{-1}$) in DMF solution in presence of metal ions.

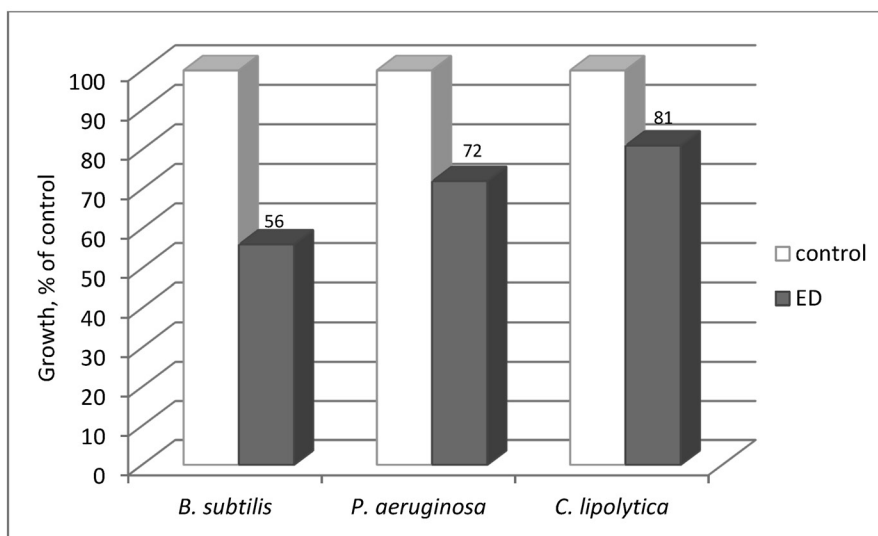


Fig. 8. The effect of ED on the growth of *B. subtilis*, *P. aeruginosa* and *C. lipolytica* tested in meat-peptone broth (MPB).

Gram-positive *B. subtilis* was slightly more sensitive than Gram-negative *P. aeruginosa*: ED decreased the growth of *B. subtilis* and *P. aeruginosa* by 44% and 28%, respectively. This could be attributed to the different outer structural arrangement of the bacterial cell walls: Gram-negative bacteria have an additional outer membrane when compared to Gram-positive bacteria. The yeasts *C. lipolytica* showed higher resistance to ED than the bacterial strains with growth reduction of 19%.

CONCLUSION

A new fluorescent PAMAM dendron has been synthesized and characterized. Its basic photophysical characteristics have been investigated in organic solvents of different polarity and it has been found solvent dependence. The influence of various metal cations (Li⁺, Na⁺, K⁺, Ag⁺, Co²⁺, Zn²⁺, Mn²⁺, Ni²⁺, Cu²⁺, Fe³⁺ and Cr³⁺) on the fluorescence intensity of the new Dendron has been investigated in DMF

solution with regard to its potential application as a fluorescent sensor for metal ions. It has been shown that the fluorescence intensity depends strongly on the nature of metal cations. Fluorescence quenching has been observed. The new compound exhibited good antimicrobial activity against Gram-positive and Gram-negative bacteria and yeasts, which makes this new PAMAM dendron interesting for biological applications.

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НОВ ФЛУОРЕСЦЕНТЕН ПАМАМ ДЕНДРОН СЪС СЕНЗОРНА И МИКРОБИОЛОГИЧНА АКТИВНОСТ

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(Резюме)

Синтезиран и охарактеризиран е нов дендрон, съдържащ еозин Y като флуоресцентен фрагмент. Изследвани са основните фотофизични характеристики на дендрона в органични разтворители с различна полярност и получените резултати са сравнени с модифициран еозин Y, използван при синтеза на флуоресцентния дендрон. В присъствието на различни по природаметални катиони (Li^+ , Na^+ , K^+ , Ag^+ , Co^{2+} , Zn^{2+} , Mn^{2+} , Ni^{2+} , Cu^{2+} , Fe^{3+} и Cr^{3+}) новосинтезираният дендрон гаси флуоресцентната си интензивност, като гасенето зависи от природата на металните катиони. Установено е, че интензитетът на флуоресценцията на дендрона не се влияе от рН на средата, за разлика от модифицирания момомерен еозин Y. Тествана е антимикробната активност на дендрона в месо-пептонен бульон спрямо някои бактерии и дрожди.