Research Article

Desislava Staneva, Stanislava Yordanova*, Evgenia Vasileva-Tonkova, Stanimir Stoyanov, Ivo Grabchev

Photophysical and antibacterial activity of light-activated quaternary eosin Y

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Abstract: The functional characteristics of a new eosin dye with biocidal quaternary ammonium group (E) were studied in aqueous solution and in organic solvents of different polarity. The spectral properties depend on the nature and polarity of the respective solvents. The antimicrobial activity of compound E has been tested in vitro against Gram-negative bacteria (*Escherichia coli*, *Acinetobacter johnsoni* and *Pseudomonas aeruginosa*), Gram-positive bacteria (*Sarcina lutea* and *Bacillus cereus*) and the antifungal activity was tested against the yeasts *Candida lipolytica* in solution and after treated on cotton fabric. Broth dilution test has been used for quantitative evaluation of the antimicrobial activity of compound E against the model strains. The ability of compound E to inhibit the growth of model Gram-negative *P. aeruginosa* strain was assessed after 16 h of incubation in presence and absence of light. These experiments were conducted in planktonic format in solution and on cotton fabric. The results suggest that the new compound is effective in treating the relevant pathogens with better results being obtained by irradiation with light. In this case the quaternary ammonium group promotes the binding of eosin Y moiety to the bacterial cell wall thus accelerating bacterial photo inactivation.

Keywords: eosin Y, photophysics, antimicrobial activity, antibacterial textile

1 Introduction

Fluorescent compounds are often used in medicine, pharmacy, biology and environmental protection [1,2]. Among the known fluorophore structures used in these fields, the eosin Y and its derivatives are very important. They belong to the group of xanthene fluorescent dyes with a wide range of photophysical and biological applications, due to their low toxicity *in vivo*, and high water solubility [3]. The utility of eosin derivatives is associated to their good spectral characteristics and the possibility to interact with different type of biomolecules [4-8]. Photophysical properties of eosin in solution strongly depend on the solvents polarity and possibility of hydrogen bond formations. Depending on pH eosin Y exhibit tautomeric structures with different proteolytic forms, and its colour depends on the respective forms [9,10]. In recent years, eosin Y was also successfully used as photoredox catalyst in organic synthesis [11,12].

A new scientific area of research is the combination of dyeing process with antibacterial properties in one compound [13]. This can be achieved through the introduction of specific groups into the dye chromophore systems to give antibacterial properties without changing their colour characteristics. In this case quaternary ammonium group can be bonded to the fluorophores by incorporating alkyl chain into the chromophore system through covalent bonds. The cationic dyes thus obtained show good colour characteristics and high antibacterial activity in solutions [14-18]. In the last years in our laboratory, fluorophores with different chemical structure having ammonium quaternary groups have been synthesized and their antibacterial and antifungal activity were investigated in solution or after their incorporation into polylactide matrix, or on the textile fabrics [19-21]. The relevance of such studies is due to the fact than in the last

*Corresponding author: Stanislava Yordanova, Sofia University "St. Kliment Ohridski", Faculty of Chemistry and Pharmacy, 1164 Sofia, Bulgaria, E-mail: SYordanova@chem.uni-sofia.bg

Desislava Staneva, University of Chemical Technology and Metallurgy, 1756 Sofia, Bulgaria

Evgenia Vasileva-Tonkova, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria
Stanimir Stoyanov, Sofia University "St. Kliment Ohridski", Faculty of Chemistry and Pharmacy, 1164 Sofia, Bulgaria

Ivo Grabchev, Sofia University "St. Kliment Ohridski", Faculty of Medicine, 1407 Sofia, Bulgaria

Scheme 1: Chemical structure of quaternary eosin Y (E).

years the antimicrobial resistance of different pathogens has become a major problem in medicine and clinical practice. This encouraged many research laboratories to start searching for and investigating novel and more active antibacterial drugs [22-24].

In the last few years, photodynamic therapy has been used against the resistance of pathogens to the medicines administered in practice. In this case, the microbiological activity of the preparations used is due to the generation of reactive oxygen species upon irradiation with visible light, which kill the bacteria by oxidative burst [25]. It has been reported that eosin Y has some antibacterial photoactivity [26].

In this paper we present the results on photophysical characterization of a new ammonium quaternary eosin Y in organic solvents of different polarity and evaluation of its antimicrobial activity against different pathogens. The effect of visible light on the antimicrobial activity of the new eosin Y derivative has also been tested in solution and after its deposition on cotton fabric.

2 Experimental part

2.1 Materials and methods

The synthesis and application of a quaternary ammonium eosin Y (E) as photoinitiator of polymerization of acrylate monomers has been described recently (Scheme 1) [27]. The light source of lamp used for irradiation was with the parameters: HL 8325, 25 w, 1230 Lumen, 6400 K, Horoz. Absorption spectra were performed using “Thermo Spectronic Unicam UV 500” spectrophotometer. The fluorescence spectra were taken on a “Cary Eclipse” spectrophotometer. All spectra were recorded using 1 cm path length synthetic quartz glass cells. Absorption and fluorescence measurements of the eosin compound E were carried out at 10^{-6} mol.L⁻¹ concentration. Organic solvents: acetonitrile (MeCN), methanol (MeOH),

ethanol (EtOH), dimethylsulfoxide (DMSO), *N,N*-dimethylformamide (DMF), dichloromethane (CH₂Cl₂), 1,4-dioxane and ethylacetate (EtOAc) used in this study were of spectroscopic grade. Fluorescence quantum yield was determined on the basis of the absorption and fluorescence spectra, using Rhodamine 6G as reference ($\Phi_{st} = 0.94$ in ethanol [28]).

2.2 Preliminary antimicrobial screening

The antibacterial activity of the investigated compound E was determined against Gram-positive bacteria (*Sarcina lutea* and *Bacillus cereus*), Gram-negative bacteria (*Acinetobacter johnsonii*, *Escherichia coli* and *Pseudomonas aeruginosa*) and the antifungal activity was tested against the yeasts *Candida lipolytica*. Microbial cultures were maintained at 4°C on Mueller-Hinton agar (MHA) slants and transferred monthly.

2.3 In vitro antimicrobial assay

Broth dilution test was used for quantitative evaluation of the antimicrobial activity of compound E against the model strains. The concentration of compound E dissolved in water was 5 mg/ml (0.623 μ M) and was further diluted in each consecutive test tube in sterile meat-peptone broth (MPB, pH 7.0) to final concentrations of 0.018, 0.038, 0.077, 0.156, 0.249 and 0.312 and 0.623 mM. After inoculation with 2% (v/v) of each standardized cell suspension, the tubes were incubated at appropriate temperature for 24 h under shaking (at 240 rpm). The microbial growth was assessed by measuring the optical density of the medium at 600 nm (OD₆₀₀). The growth control, sterility control and control of the compound E were used. The % survival of the test cultures was determined on the basis of the positive control which was considered as 100%. The experiments were conducted in triplicate. The light source used for irradiation was with the parameters: HL 8325, 25 w, 1230 Lumen, 6400 K, Horoz [27].

2.4 Antimicrobial activity of cotton fabrics

The antimicrobial effectiveness of cotton fabrics treated with 0.5% solution of the compound E was investigated by the shaking flask method. *C. lipolytica*, *B. cereus* and *A. johnsonii* were used as model strains. Test tubes containing sterile MPB (1.0 ml) and square specimens (10 mm x 10 mm) were inoculated with each overnight

grown microbial culture. Tubes with untreated cotton and without specimens were also prepared as controls. After 24 h incubation at appropriate temperature under shaking, microbial growth was determined by measuring OD_{600} . To evaluate the antimicrobial activities of the samples, the reduction in cell density between the untreated and treated samples after incubation was compared. All antimicrobial activity tests were done in triplicate.

2.5 Treatment of cotton fabrics with E

In 5 ml of water the compound **E** (5 mg) was dissolved and 1g of cotton fabric (weight 140 g/m²) was added in the solution for 30 min at 25°C. Then cotton sample was dried at ambient temperature.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and Discussion

3.1 Photophysical characteristics of ammonium quaternary eosin Y (E)

Photophysical properties of the eosin Y derivatives as a part of xanthene dyes are characterized by heterocyclic system containing a dibenzo-1,4-pyran ring. Its basic spectral characteristics depend from the solvent polarity and for formation of hydrogen bonds with solvents.

All spectral measurements of the compound **E** were investigated at a concentration of $c = 1 \times 10^{-6}$ mol.L⁻¹ and in this concentration the solution is highly coloured in red-pink colour. Table 1 presents its spectral characteristics in different organic solvents: the fluorescence (I_F) and absorption (I_A) maxima, Stokes shift ($n_A - n_F$), quantum yield of fluorescence (F_F) and the molar absorptivity (ϵ).

From the data in Table 1 it is seen that the ammonium quaternary eosin **E** has absorption maxima at 515-540 nm and the respective fluorescence maxima are at 540-562 nm. All absorption spectra exhibit bands with a well pronounced maximum and short-wavelength shoulder as it can be seen from Figure 1 as an example. Also Figure 1 shows that the fluorescence curve is approximately mirror images of the absorption curves which is typical for such structures with allowed transitions and similar geometries in excited and ground state.

The position of the absorption and fluorescence maxima depend from the polarity of solvents (Figures 2 and 3). In the case of solvents containing hydroxyl group

Table 1: Photophysical characteristics of compound **E** in solvents with different polarity.

Solvent	I_A nm	I_F nm	$n_A - n_F$ cm ⁻¹	ϵ l mol ⁻¹ cm ⁻¹	F_F
Water	515	535	726	110000	0.22
MeOH	522	540	639	110000	0.59
EtOH	529	546	589	120000	0.54
DMF	538	552	471	110000	0.36
MeCN	538	556	602	98000	0.71
DMSO	545	562	555	95100	0.68
CH ₂ Cl ₂	543	561	590	95100	0.22
EtOAc	540	556	533	94200	0.40
Dioxane	540	555	501	92100	0.20

Figure 1: Normalized absorption (black) and fluorescence (red) spectra of the compound **E** in *N,N*-dimethylformamide solution.

such as alcohols and water both absorption and emission maxima are hypsochromically shifted compared to the other solvents. The difference is due to the enhancing the dipole moment of the molecule upon excitation due to the electron density distribution and from other hand the possibility for formation of hydrogen bonds.

Stokes shift ($n_A - n_F$) is important parameters of the fluorescent compounds which indicate the difference in properties and structure between the ground S_0 and the first excited state S_1 and it has been estimated according to Equation (1):

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

Figure 2: Absorption (A) and fluorescence (B) spectra of compound **E** in solvents with different polarity.

Figure 3: Normalized excitation (black) and fluorescence (red) spectra of compound **E** loaded on the cotton fabric.

From Table 1 it is seen that the Stokes shift is in a narrow range (between 501 and 726 cm^{-1}) and it depends on the solvents. Larger values were obtained at nonpolar solvents and the results are very typical to this class of compounds [29-32].

The ability of the photoactive molecules to emit the absorbed light energy is characterized quantitatively by the fluorescence quantum yield F_F . It has been calculated on the basis of the absorption and fluorescence spectra using Rhodamine 6G as a standard according to Equation (2).

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

The calculated F_F were in the region 0.20-0.68 and its values depend on the polarity and chemical nature of the

solvents. In polar solvents the obtained yields are more than three times higher compared to these in non-polar solvents.

3.2 Absorption and fluorescence investigations of coloured cotton fabric

To investigate the antimicrobial activity of compound **E**, it has been superficially deposited on a cotton fabric, giving it intense red colour and fluorescence. Figure 3 plots the normalized excitation spectrum and fluorescence spectrum having maxima at ($\lambda = 526 \text{ nm}$) and ($\lambda = 562 \text{ nm}$) respectively. These results show that in solid state compound **E** has similar absorption wavelength value to these in alcohol solution, probably due to the fact that cotton cellulose molecules are enriched with hydroxyl groups that have a similar behaviour as the alcoholic hydroxyl groups at formation of intermolecular bonds. The fluorescence maximum is bathochromically shifted compared to that in alcohol, which can be explained by the strong fixation of the fluorophore molecule to the textile matrix and hence for the lack of conformational changes in the transition from excited S_1 to the ground state S_0 .

The release of compound **E** from the surface of cotton fabric has been measured in aqueous solution at pH = 7.2 by absorption and fluorescence spectroscopy in the condition of dropping method for 60 minutes.

Through the contact of coloured cotton fabric with water solvent the hydrophilic dye **E** is released from the surface cotton matrix to the aqueous solution, which becomes colourful. Figure 4 shows that the absorption and respective fluorescence intensity of compound **E** increase

Figure 4: Absorption (A) and fluorescence (B) release profile of E from cotton matrix in aqueous solution at pH = 7.2 at 25°C for 60 min.

with time then it reaches a plateau. It is seen that in the beginning the cotton fabric releases a large amounts of E, and accordingly the absorption and fluorescence intensity increase drastically, and with time this effect disappear. This indicates that compound E leaves the cotton fabric and passes into the aqueous solution. The respective maxima of E in this solution are $\lambda_A = 515$ nm and $\lambda_F = 535$ nm, matching these in freshly prepared water solution. The observation that there is no change of the absorption and fluorescence maxima positions during the extraction, but only the intensity is increased, give evidence that the dye didn't undergo any chemical change during the deposition and the release. This is a new important characteristic of coloured cotton fabric, which indicate that E release into the water solution exhibiting a prolonged antimicrobial activity.

4 Antimicrobial activity

4.1 Growth inhibitory activity in aqueous solution

A quantitative evaluation of the antimicrobial activity of the synthesized compound was carried out by the shaking flask test against two Gram-positive bacteria (*B. cereus*, *S. lutea*), three Gram-negative bacteria *P. aeruginosa*, *A. johnsonii*, *E. coli*) and the yeasts *C. lipolytica*. Figure 5 shows changes in the growth of the strains in presence of different concentrations of E ranging from 0.018 μ M to 0.623 μ M. As can be seen, the compound E reduced the growth of all test cultures with increasing of its concentrations as compared to the negative control. The relative order of

sensitivity to the compound was found to be a function of the strain. The compound exhibited highest antimicrobial efficiency against the test Gram-positive bacterium *S. lutea* and the yeasts *C. lipolytica* (MICs at 0.156 μ M) followed by Gram-negative bacterium *A. johnsonii* (MIC at 0.249 μ M) and Gram-positive *B. cereus* (MIC at 0.312 μ M). *P. aeruginosa* and *E. coli* exhibited highest resistance to the compound E than the other cultures and MICs were not reached up to 0.623 μ M.

4.2 Antimicrobial activity of modified cotton fabric

The antimicrobial activity of cotton fabric treated with E has been evaluated by the reduction in bacterial growth. Gram-positive *B. cereus*, Gram-negative *A. johnsonii*, and the yeasts *C. lipolytica* were used as model strains. It was found that the treated cotton textile leads to slight reduction of the growth of *B. cereus* and *C. lipolytica* by about 14% and 22%, respectively, and no growth reduction of *A. johnsonii* was observed (Figure 6) In this case the slow release of compound E from the cotton matrix into the aqueous medium, and direct contact with matogenic cells contributed to the antimicrobial effect of the modified cotton fabric.

5 Effect of light irradiation on bacterial growth

The ability of E to inhibit the growth of model Gram-negative *P. aeruginosa* strain was assessed after 16 h

Figure 5: Growth of the tested model microbial strains in presence of different concentrations of compound E.

Figure 6: Effect of cotton fabric treated with compound E on growth development (quantified by OD at 600 nm) of the indicated model microbial strains.

of incubation in presence and absence of light. The experiments were conducted in planktonic format in solution and applied on cotton fabric. In solution, without illumination, we observed higher density of *P. aeruginosa* cells compared to the illuminated sample (Figure 7). With light irradiation, the antibacterial effect was significantly higher at concentration of eosin Y 0.25 μM than observed for the 0.125 μM concentration. In the experiments with eosin-treated and non-treated cotton fabrics, about 41% reduction of cell density of *P. aeruginosa* was established in the absence of illumination, while almost complete growth inhibition was observed in the illuminated sample (Figure 8). Similarly to some findings reported previously [33, 34], it can be assumed that eosin Y produce large amount of singlet oxygen near the outer membrane of bacteria leading to membrane damage. Quaternary ammonium group promotes the binding of eosin Y moiety to the bacterial cell wall thus accelerating bacterial photo-inactivation.

Figure 7: Effect of visible light on planktonic cultures of *P. aeruginosa* in presence of compound E.

Figure 8: Effect of light irradiation on the antibacterial effect of cotton fabric treated with compound E.

6 Conclusions

The photophysical characteristics of a new eosin Y functionalised with quaternary ammonium biocidal group have been investigated in different media. The results demonstrated that the modified eosin Y exhibits intense fluorescence in solutions which was retained after its deposition on the surface of cotton fabric. The results showed good inhibitory activity of the novel eosin compound E towards the tested microbial cultures. Antimicrobial activity of cotton fabric treated with the new eosin derivative E was investigated against the strains *A. johnsonii*, *B. cereus* and *C. lipolytica*. The results showed that the compound E has released slowly into the aqueous solution and exhibits a prolonged antimicrobial activity. The modified cotton fabric exhibited higher bioactivity against *B. cereus* and *C. lipolytica*, suggesting its suitability for application as a new additive in preparation of antibacterial textile fabric. The new compound E can be used for the photodynamic bacterial inactivation.

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