Chemical modification and characterization of cotton fabric with 1,8-naphthalimide and its antibacterial activity

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Abstract. 1,8-naphthalimide (NI) containing pyridinium nuclei attached by a methylene bridge to the imide nitrogen atom or at the C-4 atom has been obtained by a single-step synthesis. The main photophysical characteristics of NI in organic solvents and water have been studied. A cotton fabric has been modified with chloroacetyl chloride. The possibility of chemical binding of NI to the modified cotton fabric, by quaternization of the pyridinium nuclei and by physical deposition and retention of NI to the surface of the cotton fabric was investigated. The antimicrobial test showed good activity of NI against Gram-positive and Gram-negative bacteria and a fungal strain of the genus Candida, which is preserved after its deposition on cotton fabric. It has been shown that covalent bonding contributed for a better activity compared to the physical deposition and retention of NI on the surface of the cotton fabric.

1. Introduction

In recent years, there has been an increased resistance of microorganisms to antibiotics used in clinical practice [1,2]. Despite the wide range of modern antibiotics and new approaches to combating this resistance, recurrent infections remain a problem for human health and quality of life. This requires searching for and finding effective compounds exhibiting antibacterial activity.

1,8-naphthalimide are a special class of heterocyclic fluorophores that emit blue or yellow-green fluorescence. They are used as dyes for polymeric materials and textiles [3], in the design of sensor systems for the detection of metal ions and protons [4]. Also some 1,8-naphthalimides derivatives of are of the wide biomedical interest due to their high antibacterial, antifungal, anti-inflammatory, antiviral, or antitumor activity [5–7]. The color and fluorescence characteristics of the 1,8-naphthalimides depend on the polarity of the chromophore system. In the presence of electron donor substituents, such as amino derivatives, their color is yellow, which is further enhanced by the emitted yellow-green fluorescence. This color is preserved even after their deposition on various textile or polymeric materials which makes them suitable for dyeing in order to give them an intense color [3].
It is of great interest to combine the properties of some 1,8-naphthalimides, which have shown good microbiological activity with their ability to dye textile materials, thus handing them antibacterial or antifungal activity [8-10].

The aim of this study was to synthesize a 1,8-naphthalimide with pyridinium units able to form chemical bonds with modified cotton fabric with chloroacetyl chloride. The antimicrobial activity against model Gram-positive and Gram-negative bacteria and yeasts in solution and after deposition on a cotton fabric has also been investigated.

2. Experimental part

2.1. Materials and methods

Thermo Spectronic Unicam UV 500 spectrophotometer and “Cary Eclipse” spectrofluorometer have been used for photopysycal measurements. The spectra were recorded using a 1 cm path length synthetic quartz glass cell at concentration $10^{-5}$ mol L$^{-1}$. The fluorescence quantum yield ($\Phi_F$) has been calculated on the basis of the absorption and fluorescence spectra by equation 1.

$$\Phi_F = \Phi_u \frac{S_u A_u n_{Du}^2}{S_a A_a n_{Ds}^2}$$

where the $\Phi_F$ is the emission quantum yield of the sample; $\Phi_u$ is the quantum yield of the standard Rhodamine ($\Phi_{ref} = 0.94$ [11]); $A_u$ and $A_a$ are the absorbance of the standard and sample at the excited wavelength, respectively; while $S_u$ and $S_a$ are the integrated emission band areas of the standard and sample, at the excited wavelength respectively, and $n_u$ and $n_s$ is the solvent refractive index of the standard and sample; subscripts $u$ and $s$ refer to the unknown and standard, respectively. All organic solvents are of spectroscopy grade: dioxane; tetrahydrofurane (THF); chloroform; acetonitrile; N,N-dimethylformamide (DMF); dimethylsulfoxide (DMSO); ethanol (EtOH); methanol (MeOH); water.

$1^H$ NMR (600.13 MHz) and $13^C$ (150.92 MHz) spectra were acquired on an AVANCE AV600 II+ NMR spectrometer. Fourier-transform spectrometer (IRAffinity-1 Shimadzu, Kyoto, Japan) at a 2 cm$^{-1}$ resolution. The color coordinates ($L^*a^*b^*$, XYZ and $xy$) of dyed cotton fabrics have been determined by using Datacolor Spectraflash SF300 spectrophotometer (Datacolor, NJ, USA). The bifunctional chloroacetyl chloride (CAC) has been used without further purification as obtained from Sigma-Aldrich.

2.2. Synthesis of 2-(pyridin-4-ylmethyl)-6-((pyridin-4-ylmethyl)amino)-1H-benzo[de]isoquinoline-1,3(2H)-dione (NI)

4-nitro-1,8-naphthalic anhydride (2.43 g, 0.01mol) was dissolved in 50 ml DMF and 4-(aminomethyl)pyridine (2.5 ml, 0.025 mol) was added. The mixture was stirred 6 hours, at 80°C then poured into 200 ml of water. The precipitate was filtered, washed with water and dried under vacuum. Yield: 3.51g, 89 %;

FT-IR (KBr) cm$^{-1}$: 3274, 3064, 2967, 2837, 2790, 1674, 1636, 1581, 1551, 1386, 1364, 1244, 1123, 1018, 775, 751; $1^H$ NMR (CDCl$_3$, $\delta$, ppm): 8.55 (d; $J=7.36$ Hz; 4H); 8.48 (d; $J=8.22$ Hz; 1H); 8.26 (d; $J=8.09$ Hz; 1H); 8.04 (t, $J=6.6$ Hz; 1H); 7.40 (d, $J=8.11$ Hz; 1H); 4.44 (s; 1H); 4.30 (s; 1H); $1^C$ NMR (CDCl$_3$, $\delta$, ppm): 163.7, 163.1, 150.3, 145.3, 144.1, 131.2, 130.3, 128.4, 127.4, 125.9, 113.3, 110.0, 45.1;

Elemental analysis: C$_{20}$H$_{18}$NO$_2$ (394.21 g mol$^{-1}$): Calc. (%): C 73.06, H 6.20, N 13.03; Found (%): C 70.43, H 6.13, N 13.09.

2.3. Modification of cotton fabric with chloroacetyl chloride [10]

1.0 g of cotton fabric (140 g / m$^2$) is placed in a beaker and soaked in DMF. At room temperature for 30 minutes, chloroacetyl chloride 10% (v/v) dissolved in DMF was added and stirred for 90 minutes, after which the cotton fabric has been removed, washed thoroughly with water and dried. The solution was of a liquor to goods ratio of 20:1.
2.4. Dyeing of cotton fabric with NI (Cotton1-NI)
1.0 g of cotton fabric (140 g / m²) was immersed into the NI (5 mg dissolved in 10 ml DMF) at 25°C. After dyeing for 30 min, the sample was washed with water and dried at room temperature.

2.5. Dyeing of modified cotton fabric with NI (Cotton2-NI)
1.0 g of the modified cotton fabric has been placed in a flask and 20 ml of DMF was added. Heat to 80°C and over 30 minutes and a solution of 5 mg of NI dissolved in 5 ml of DMF was added. Under these conditions for 6 hours has been stirred, then the colored cotton fabric has been washed thoroughly with water. The unreacted dye is removed by the boiling of cotton fabric in ethanol. All future measurements and tests were performed on this sample.

2.6. Antimicrobial activity test

2.6.1. Minimum Inhibitory Concentration (MIC)
Broth dilution test was used for determination of the MICs of NI against Gram (+) B. cereus and Gram (-) P. aeruginosa as test cultures. The compound was serially diluted in tubes with meat-peptone broth (MPB) in concentrations in the range 20 - 1000 µg/ml. Control tubes without added compounds were also prepared for each microbial culture. The tubes were inoculated with respective microbial suspension and incubated at appropriate temperature for 24 h. Microbial growth was assessed by the turbidity at 600 nm (OD₆₀₀). The growth control, sterility control and sample control were used. The lowest concentration of the samples that inhibited the visible growth of the strains was referred as MIC. Three independent experiments were carried out and averages are given.

2.6.2. Antibacterial activity of modified cotton fabrics
Modified with NI cotton fabrics was tested for antibacterial activity against B. cereus and P. aeruginosa as model strains. Test tubes containing MPB and square cotton specimens (1 cm x 1 cm, 25 mg) were inoculated with cell suspension of each bacterial culture. Tubes with untreated cotton and without specimens were also prepared as controls. After incubation for 24h at appropriate temperature, the specimens were removed and OD₆₀₀ was determined. The antimicrobial activity of the treated cotton samples was evaluated by the reduction of OD₆₀₀ after incubation compared to the control sample. All antimicrobial tests were done in triplicate and the average was taken; standard deviations were less than 5%.

3. Results and discussion

1,8-naphthalimide (NI) has been obtained in a single-step synthetic route as shown in Scheme 1. 4-Nitronaphthalic anhydride was reacted with 4-(aminomethyl)pyridine in a DMF medium at 80°C to simultaneously nucleophilically replacement the nitro group at C-4 with 4-(aminomethyl)pyridine and condensation of the primary amino group in the anhydride ring. Thus, 1,8-naphthalimide containing two pyridinium nuclei has been synthesised. The choice of these substituents has been dictated by the fact that the pyridinium core can be quaternized with the residues of the acylated cotton fabric, and thus to form a covalent bond of NI with the cotton matrix.
In order to obtain a yellow colored cotton fabric with microbiological activity, 1,8-naphthalimide, has been used as dye using two approaches. In the first method, unmodified cotton fabric was used, and NI was physically deposited by irrigation at room temperature for 30 minutes, then washed with water and dried. In this way, NI is deposited on the cotton fabric, and the way it is retained on the surface is mainly through hydrogen bonds and van der Waals forces of attraction. Poor solubility in the water further aids its retention (Cotton1–NI).

In the second case, a pre-modified with chloroacetyl chloride cotton fabric has used [10]. It has been used due to the different degree of activity of the two chlorine atoms. The much more reactive acyl chlorine atom reacts with the hydroxyl groups of the cotton, leaving a second reactive chlorine atom. 1,8-naphthalimide under study has two pyridinium nuclei in its structure that can participate in a quaternization reaction with alkyl chlorine substituents (Scheme 2). The quaternization reaction has performed under DMF at 80 °C for 6 hours. After boiled in ethanol for 30 minutes, the color of the fabric remains yellow, which means that NI has covalently bound to the cotton fabric (Cotton2–NI).

The basic photophysical characteristics of NI have been studied in organic solvents with different polarities and water (Table 1). NI is yellow in the solvents tested. Its absorption maxima are in the spectral range $\lambda = 412-436$ nm, and the emitted fluorescence has maxima at $\lambda = 495-536$ nm. Figure 1 shows the dependence of the position of these maxima on the polarity of the medium (ET30). The results show that as the polarity of the solvents increases, the absorption and fluorescence maxima are shifted batochromically, and NI exhibits positive solvatochromism. The Stokes shift ($\nu_A-\nu_F$) values are in the range 3730-4170 cm$^{-1}$, which are typical of 1,8-naphthalimide derivatives with alkylamino substituents at C-4 [12,13].

| Solvents | $\lambda_A$ (nm) | $\lambda_F$ (nm) | $\nu_A-\nu_F$ (cm$^{-1}$) | $\varepsilon$ (mol l cm$^{-1}$) | $\Phi_F$ |
|----------|-----------------|-----------------|--------------------------|--------------------------|----------|
| DMF      | 432             | 515             | 3730                     | 8400                     | 0.26     |
| Water    | 438             | 536             | 4170                     | 8160                     | 0.12     |
Etanol & 437 & 519 & 3620 & 9790 & 0.32  
Dioxane & 412 & 495 & 4070 & 8460 & 0.32  
Methanol & 437 & 523 & 3760 & 8550 & 0.25  
Chloroform & 415 & 501 & 4140 & 8450 & 0.30  
DMSO & 435 & 520 & 3760 & 7440 & 0.24  
THF & 421 & 498 & 3670 & 8210 & 0.27  
Acetonitrile & 422 & 509 & 4050 & 8610 & 0.28  

The quantum efficiency of NI has been determined by calculating the quantum yield of fluorescence. The data in Table 1 show that for organic solvents the PF is in the range $\Phi_F = 0.24$-$0.32$, which means that the polarity of the medium does not significantly affect this indicator. A significantly lower value was obtained in aqueous solution ($\Phi_F = 0.12$). In this case, in addition to the polarity of the medium, the dipole-dipole interaction between water molecules and NI also has an effect, which leads to a decrease in the polarization of the chromophore structure.

Fig. 1. Dependence of absorption and fluorescence maxima of NI2 on the empirical parameter of solvent polarity $E_{T}(30)$: 1-dioxane; 2-THF; 3-chloroform; 4-acetonitrile; 5-DMF; 6-DMSO; 7-EtOH; 8-MeOH; 9-water (A) and fluorescence spectra of NI at the same solvents (B).

Figure 2. Excitation (Ex) and fluorescence (Fl) spectra of cotton fabrics
The excitation and fluorescence spectra of the colored cotton fabrics obtained by both methods were studied (Figure 3) and the corresponding maxima were determined. It was found that at Cotton1–NI the maxima are at 462 nm and 527 nm, while in Cotton2–NI they are at 456 nm and 527 nm, respectively, but the fluorescence intensity is almost twice high. This difference can be explained by the quaternization of the nitrogen atoms of the pyridinium structure in the formation of the chemical bond with the cotton fabric.

Figure 3. Micrograph of non-treated cotton fabrics (Control) and treated with dendrimer Cotton1–NI and Cotton2–NI upon their irradiation with Sunlight and UV light

Figure 3 shows the change in the color of Cotton1–NI and Cotton2–NI cotton fabrics compared to that of the untreated cotton fabric during the irradiation with UV light (λmax = 365 nm) and daylight. The untreated cotton fabric has a white color in daylight. Also it is seen that, when irradiated with daylight the treated cotton fabrics have a saturated yellow color, while when irradiated with UV light the color changes to yellow-green.

The chromaticity coordinates (x and y) and CIEab coordinates (L*, a* and b*) of both cotton fabric has been determined by Datacolor systems using equations 2-4 [14]:

\[
L^* = 116 \left( \frac{Y}{Y_0} \right)^{1/3} - 16
\]

\[
a^* = 500 \left( \frac{X}{X_0} \right)^{1/3} - \left( \frac{Y}{Y_0} \right)^{1/3}
\]

\[
b^* = 200 \left( \frac{Y}{Y_0} \right)^{1/3} - \left( \frac{Z}{Z_0} \right)^{1/3}
\]

were the tristimulus values of specified achromatic light used in illumination are X₀, Y₀, Z₀. The values of Y₀ is normalized in such a way that Y₀ = 100 and the respective X, Y, Z coordinates has been determine. As a standard has been used a non-treated cotton fabric. The date obtained are summarised in Table 2. The results show that the dyed cotton fabrics have a yellow color.

| Table 2. Color characteristics of non-treated cotton fabric and treated with NI |
|-----------------|-----|-----|-----|-----|-----|-----|-----|
| L*  | a*  | b*  | X   | Y   | Z   | x   | y   |
| Non treated cotton | 93.77 | -0.26 | 3.75 | 80.22 | 84.75 | 85.65 | 0.3201 | 0.3382 |
| Cotton1–NI          | 86.76 | 0.16 | 24.09 | 65.99 | 69.52 | 48.13 | 0.3593 | 0.3786 |
| Cotton2–NI          | 88.07 | -3.11 | 46.06 | 67.06 | 72.22 | 31.83 | 0.3919 | 0.4221 |
The results show that the way of connecting the NI to the cotton matrix affects the color characteristics of the fabric. In both cases the color of the cotton fabric is yellow but with a different shade. In covalent bonding, it has a deeper color but is more brilliant due to the emission of the more intense fluorescence. This difference can be explained by the quaternization of the pyridinium nuclei, as that is in the C-4 position will have a greater influence.

The antimicrobial activity of NI has been investigated against Gram-positive bacteria *B. cereus*, Gram-negative bacteria *P. aeruginosa*, and the fungus *C. lipolytica*. The MICs of the compounds against the model strains have been determined in MPB. The inhibition of the growth of Gram-positive *B. cereus* was higher (the lowest MIC, 250 mg/ml; 0.63 mM) while in *C. lipolytica* the activity is approximately two-fold lower (MIC - 1.40 mM). Significantly lower activity has been determined against Gram-negative *P. aeruginosa* (MIC at 1.01 mM). Based on classification of Holetz et. al. antimicrobial activity of the investigated compounds can be considered moderate [15].

Various methods have been proposed for the antimicrobial modification of material surfaces as an alternative way of preventing the formation of highly resistant biofilms [16]. The antimicrobial effect of the treated with NI cotton fabrics has been evaluated by the reduction of the growth of the model bacteria Gram-positive *B. cereus* and Gram-negative *P. aeruginosa* bacteria. Against *P. aeruginosa*, both cotton samples showed similar reduction of the growth (5-10%) (Figure 4). The low activity in this case is due to the fact that NI is firmly attached to the cotton surface, which does not allow to diffuse from the fabric into the solution. This significantly reduces the possibility of direct contact of bacteria with NI. Against *B. cereus*, the activity was better expressed in Cotton2-NI (30%) and 10% in Cotton1-NI. Probably in this case the difference is due to the presence of quaternary groups, which more easily damage the membrane of Gram-positive bacterial cells.

![Figure 4. Reduction of the growth of model bacteria by treated cotton fabrics](image)

4. Conclusion

By one-step synthesis, 1,8-naphthalimide has been obtained, containing pyridinium nucleattached to the imide nitrogen atom and to C-4 via methylene (-CH2-) spacer. A cotton fabric was dyed by two methods, in which NI is retained on the surface of the fabric by physical forces of attraction or by forming a strong covalent bond. In the second case, the cotton fabric is modified with chloroacetyl chloride and then it interacts with NI. The color parameters of both cotton fabrics were determined and it was shown that a brighter yellow color is obtained with covalently bonded NI. The photophysical characteristics of NI in various organic solvents have been studied and it has been shown that in solution it has an intense yellow color and fluorescence, which are preserved even after its application on a cotton fabric. The
fluorescence intensity is almost twice as high in the case where the NI is covalently bonded to the cotton fabric. NI showed moderate activity against the tested cultures, better expressed against Gram-positive bacteria. The antibacterial activity of the stained fabrics was studied and found to be more pronounced in covalent binding of NI.

Acknowledgments
This work was supported by Grants KPI-06-KOCT/19, Fund Scientific Research, Ministry of Education and Science of Bulgaria.

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