Synthesis and characterisation of a new water soluble fluorescent cationic polymer and its microbiological activity

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Abstract. The work describes the synthesis of a new fluorescent cationic polymer (PNI) containing 1,8-naphthalimide units. Its photophysical characteristics have studied in organic solvents and water. The investigations have revealed, the polymer in solution to have an intense yellow color and to emit yellow-green fluorescence. These properties have been preserved after depositing it onto a cotton fabric. The color coordinates (L*, a*, b* and XYZ) of the dyed cotton fabric have been determined. The antimicrobial activity of PNI has been studied in a liquid medium against bacterial strains Bacillus cereus and Pseudomonas aeruginosa, and the fungal strain Candida lypolitica. The results obtained show moderate activity of the polymer against the tested strains which had been preserved after its loading onto the cotton fabric.

1. Introduction
Due to the compact molecular structure and low molecular weight, 4-amino substituted 1,8-naphthalimide fluorophores are one of the most studied organic fluorophores with intense yellow-green color for dyeing textile and polymeric materials [1]. In this case, the nuance of the color and fluorescence intensity depends on the polarization of the chromophore system, which is predetermined by the electron-donor capacity of the substituent at the C4 position. Thus, by varying the type of substituents, fluorophores with tailored properties can be obtained [2-6]. On the other hand, by introducing special substituents attached to the imide nitrogen atom and to the C4 atom of the chromophore system, 1,8-naphthalimide derivatives can be used as fluorophores for structural coloring of polymeric materials. Thus they are covalently attached to the polymer chain and no migration from the surface or inside of the polymers is observed [1]. Derivatives of 1,8-naphthalimide can also participate in polycondensation processes with some traditional monomers [1]. Polypropylene imine and polyamidoamine dendrimers modified with 1,8-naphthalimide units acquire interesting photophysical characteristics and can be used in the design of highly efficient sensor systems for detecting metal ions and protons [7-10].
In recent years, the antimicrobial activity of 1,8-naphthalimide derivatives has been intensively studied [11-14]. The incorporation of such compounds into polymer or dendrimer macromolecules leads to an increase in their biological activity [15-17]. This activity is preserved after their deposition onto textile materials, which gives them new valuable properties [18,19]. Such textiles with antibacterial properties are widely used in for domestic purposes, industry, automotive plants in order to reduce the growth of pathogenic bacteria. Their application in clinical practice for the production of bed linen, wound dressings, surgical sutures, etc. has been especially relevant.

The aim of this work is to synthesize a new polymer with quaternary groups and to study its photophysical characteristics in organic solvents. The antimicrobial activity of the polymer and the colored cotton fabric have also been assessed.

2. Experimental part
2.1. Materials and methods
The synthesis and characterization of the initial monomer unit: 2-(pyridin-4-ylmethyl)-6-((pyridin-4-ylmethyl)amino)-1H-benzo[de] isoquinoline-1,3(2H)-dione (NI) was described in [20]. Organic solvents of spectroscopic grade (dioxane; chloroform; acetonitrile; dimethylsulfoxide (DMSO); N,N-dimethylformamide (DMF); tetrahydrafuran (THF); ethanol (EtOH) and methanol (MeOH)); and and sebacoyl chloride (99%) were used as obtained from Sigma Aldrich.

The photophysical measurements were performed on a Thermo Spectronic Unicam UV 500 spectrophotometer and on a Cary Eclipse spectrofluorometer. Thin layer chromatography was carried out on silica gel (Fluka F60 254 20x20; 0.2 mm) using an eluent system acetone/n-heptane (1:1). The molecular weight of polymer was determined on a GPC Water 244 apparatus with polystyrene standard and THF as an eluent. Both a differential refractive index and an UV-vis absorption detector ($\lambda_A = 430$ nm) were used. 1H NMR (600.13 MHz) and 13C (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. Color coordinates of the dyed cotton fabrics were determined using Datacolor Spectraflash SF300 spectrophotometer (Datacolor, NJ, USA).

2.2. Synthesis of Polymer (PNI)
NI (3.94 g, 0.01mol) was dissolved in 100 ml anhydrous acetone and sebacoyl chloride (4.80 ml, 0.02 mol) was added dropwise to the solution at 5°C. The mixture was stirred for 30 minutes. Then the precipitate was filtered and washed with acetone (3 times 50 ml). Yield: 8.50 g

FT-IR (KBr) cm$^{-1}$: 3222, 3072, 3066, 2993, 2944, 1683, 1648, 1633, 1575, 1503, 1356, 1299, 1240, 998, 776, 755. 1H NMR (CDCl$_3$, δ, ppm): 8.35 (d; J=7.12 Hz; 4H); 8.28 (d; J=8.12 Hz; 1H); 8.20 (d; J=8.05 Hz; 1H); 7.93. (bs; 1H); 7.54 (d, J=8.14 Hz; 4H); 7.54 (d, J=8.14 Hz; 4H); 7.20 (d, J=8.32 Hz; 1H); 7.06 (s, 1H); 4.30 (s; 1H); 2.20 (t, J=6.19 Hz; 4H); 2.02 (m, 8H); 140(m, 8H); 13 C NMR (CDCl$_3$, δ, ppm): 176.4, 164.5, 163.9, 154.3, 143.2, 140.3, 137.1, 134.2, 133.0, 130.5, 128.4, 123.4, 118.3, 48.0, 42.1, 29.3. 28.1.

2.3. Dyeing of cotton samples with PNI
1.0 g of cotton fabric (140 g/m$^2$) was immersed into 10 ml aqueous solution of PNI (10 mg) at 25°C for 30 min. After that the sample was dried at room temperature.

2.4. Antimicrobial activity test
2.4.1. Minimum Inhibitory Concentration.

Broth dilution test was used for determination of the minimum inhibitory concentrations (MICs) of PNI against Gram-positive B. cereus and Gram-negative P. aeruginosa and the yeasts C. lypolitica. The polymer was serially diluted in tubes with meat-peptone broth (MPB). The tubes were inoculated with respective microbial suspension and incubated at appropriate temperature for 24 h. The microbial growth was assessed by the turbidity at 600 nm (OD$_{600}$). 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.

2.4.2. Antibacterial activity of the treated cotton fabric.

The antibacterial activity of cotton fabric treated with PNI was tested against *B. cereus* and *P. aeruginosa* as model strains. Test tubes containing MPB and square cotton specimens (10 mm x 10 mm) were inoculated with cell suspension of each bacterial culture. Tubes with untreated cotton and without specimens were also prepared as controls. After incubation for 24 h, the specimens were removed and OD<sub>600</sub> was determined. The antimicrobial activity of the treated cotton samples was evaluated by the reduction of OD<sub>600</sub> 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

A new fluorescent polymer with quaternary ammonium groups has been obtained by a reaction between NI containing two pyridinium nuclei and a bifunctional sebacoyl chloride. In the reaction between them, the acyl chloride attacks the pyridinium nitrogen atom to form a quaternary ammonium group (Scheme 1). The reaction between the co-monomers was carried out at 5°C for 30 minutes, whereby the polymer PNI was obtained in almost quantitative yield as a bright yellow solid.

The molecular weight of the polymer Mn = 8 940 Da and the polydispersity Mw/Mn = 1.54 were determined. On the basis of the molecular weight, the degree of polymerization DP has been calculated as a ratio of molecular weight of the polymer (Mn) and molecular weight of the repeat units in the polymer structure (Mo) according to the formula DP=Mn/Mo. The results obtained have showed that, approximately eight NI units are linked in the polymer structure.

![Scheme 1. Synthesis of fluorescent PNI](image)
Figure 1 presents comparatively the IR spectra of the initial NI and the resulting polymer PNI. The characteristic bands for the carbonyl groups of NI are at 1674 cm\(^{-1}\) and 1636 cm\(^{-1}\), while for PNI they are slightly hypsochromically shifted and are at 1683 cm\(^{-1}\) and 1638 cm\(^{-1}\), respectively. That can be explained with the presence of a positive charge at the nitrogen atom of the pyridine nuclei. There is a broadening of the signal and a shoulder at 1662 cm\(^{-1}\) in the spectrum of PNI, what is due to the oscillation of the N-C = O group as this signal overlaps with the intense peak of C = O. An intense peak has been registered at 1501 cm\(^{-1}\), which refers to the oscillation of the –CH\(_2\)– groups from the polymer structure. As seen from Figure 1, this peak is missing in the spectrum of NI.

![Figure 1. Infrared spectra of NI and PNI](image)

**Table 1. Photophysical characteristics of PNI in different solvents**

| Solvents    | \(\lambda_A\) nm | \(\lambda_F\) nm | \(\nu_A-\nu_F\) cm\(^{-1}\) | \(\Phi_F\) |
|-------------|------------------|------------------|----------------------------|-----------|
| DMF         | 430              | 515              | 3934                       | 0.38      |
| Water       | 437              | 533              | 4122                       | 0.15      |
| Ethanol     | 430              | 519              | 3988                       | 0.36      |
| Dioxane     | 414              | 498              | 3505                       | 0.26      |
| Methanol    | 428              | 523              | 4244                       | 0.29      |
| Chloroform  | 424              | 502              | 5142                       | 0.25      |
| DMSO        | 425              | 511              | 3868                       | 0.22      |
| THF         | 417              | 502              | 4060                       | 0.26      |
| Acetonitrile| 415              | 508              | 4411                       | 0.24      |

The main photophysical characteristics of PNI have been studied in organic solvents of different nature and polarity. The presence of quaternary groups and positive charges in the structure of the PNI allows this polymer to dissolve well in water. In the solvents used PNI has an intense yellow color and the emitted fluorescence is yellow-green. The data in Table 1 show that, the absorption maxima are in the 415-437 nm range and the corresponding fluorescent maxima are at 498-533 nm. Figure 2A shows the dependence of these maxima on the polarity of the medium determined using the parameter \(E_{r(30)}\). The maxima are batohromically shifted in both cases comparing those in the polar environment to the ones in non polar, which means that PNI exhibits positive solvatochromism.
The fluorescence quantum yield (Φ<sub>F</sub>) has been calculated on the basis of the absorption and fluorescence spectra by equation 1.

\[
Φ_F = Φ_s \frac{S_u}{S_s} \frac{A_u}{A_s} \frac{n_{Du}^2}{n_{Ds}^2}
\]

where the Φ<sub>F</sub> is the emission quantum yield of the sample; Φ<sub>s</sub> is the quantum yield of the standard Rhodamine (Φ<sub>ref</sub> = 0.94 [21]); A<sub>s</sub> and A<sub>u</sub> are the absorbance of the standard and sample at the excited wavelength, respectively; while S<sub>s</sub> and S<sub>u</sub> are the integrated emission band areas of the standard and sample, at the excited wavelength respectively, and n<sub>s</sub> and n<sub>u</sub> are the solvent refractive indexes of the standard and sample; subscripts u and s refer to the unknown and standard, respectively.

As Figure 2B shows, the fluorescence intensity also depends on the polarity of the medium and on its ability to form hydrogen bonds with the NI chromophore system. Higher intensity has been registered in the polar solvents, where the dipole-dipole interactions and the formed hydrogen bonds lead to a change in the polarization of the NI. The calculated quantum yield of fluorescence shows that, the polarity of the medium affects also its values. In organic solvents Φ<sub>F</sub> is in the 0.22-0.38 region, while in aqueous solution it is 0.15.

The deposition of PNI on a cotton surface has been carried out after soaking the fabric with 1% aqueous polymer solution at room temperature for 30 minutes. The cotton fabric dyed with PNI is yellow in color and emits yellow-green fluorescence. The degree of loading has been determine by fluorescence spectroscopy and it has been found that, 89% of PNI used had been deposited onto the cotton surface. When PNI is deposited on the cotton fabric, the polymer is retained by the formation of hydrogen bonds and other weak non-covalent interactions between the fabrics and polymer. In this case, the hydrogen bonds have been formed with the unshared electron pairs of the carbonyl groups of the polymer and the hydroxyl groups of the cellulose structure of the cotton materials.

The color coordinates have been determined using Datacolor system: CIEab coordinates (L*, a* and b*) and chromaticity coordinates (XYZ, and xy), which have been compared with those of the untreated cotton fabric. The data obtained are summarized in Table 2. The results show that, the cotton fabric used is white, and acquires a saturated and bright yellow color after been traded with PNI.

Table 2. Color characteristics of the non-treated and treated with PNI cotton fabric

|       | L*  | a*  | b*  | X   | Y   | Z   | x   | y   |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|

Figure 2. (A) Dependence of absorption and fluorescence maxima of PNI on the empirical parameter of solvent polarity ET(30): 1-dioxane; 2-chloroform; 3-THF; 4-acetonitrile; 5-DMSO; 6-DMF; 7-Ethanol; 8-Methanol; 9-water and (B) fluorescence spectra of NI in the same solvents.
Non treated cotton  |  93.77  |  -0.26  |  3.75  |  80.22  |  84.75  |  85.65  |  0.3201  |  0.3382  
Cotton–PNI          |  77.64  |  6.73   |  57.94  |  52.42  |  52.61  |  14.88  |  0.4372  |  0.4388  

Figure 3. Fluorescence spectra (A) and change in the fluorescence intensity (B) over time after immersion of cotton–PNI into an aqueous solution

When the cotton fabric is immersed into an aqueous solution the polymer is slowly released from the surface of the cotton matrix, due to the very good water solubility of PNI. The release process has been registered by fluorescence spectroscopy. Figure 3 shows the fluorescence spectra tracking the release of PNI with the time of up to 12,000 sec. (Figure 3A) and the dependence of their intensity on time (Figure 3B). Up to 1200 seconds the release is more intense, then up to 12,000 sec., it slows down.

The antimicrobial activity of PNI has been tested against Gram-positive bacteria, Gram-negative bacteria and fungal Candida strain. The method of dilution in the concentration range $2\times10^{-6}$ - $1\times10^{-3}$M in MPB has been used to determine MICs of PNI. It has been found that, the inhibition of the growth of Gram-positive bacteria B. cereus is higher (MIC = 33.3 μM) than that of Gram-negative bacteria P. aeruginosa (MIC=44.7 μM), while in fungal strain C. lipolytica the activity was lower (MIC = 56.0 μM). The difference in the activity of PNI against the tested bacteria can be explained with the different structure of Gram-positive and Gram-negative bacteria.

The antibacterial activity of cotton fabrics treated with PNI (10/10 mm) against P. aeruginosa and B. cereus has been tested after immersion of the cotton sample into MPB. Figure 4 shows that, the reduction in bacterial growth is better expressed in the case of Gram-positive B. cereus, where the reduction is about 26%, while in Gram-negative bacteria it is 11%. In this case, the effect is due to the possibility of PNI to release from the fabric, what facilitates its direct contact with bacterial cells.
Figure 4. Reduction of the growth of model bacteria by treated with PNI cotton fabrics.

4. Conclusion
A new water-soluble cationic polymer, which emits intense yellow-green fluorescence has been obtained and characterized. The determined photophysical characteristics in organic solvents and water have demonstrated the absorption maxima to be in the 415-437 nm range and the corresponding fluorescent maxima in the 498-533 nm range with a well-defined positive solvatochromism. The quantum yield in organic solvents is in the range 0.22-0.38, while in aqueous solution it is lower. The microbiological activity of the polymer in solution and after its application onto a cotton fabric has been studied. It has been found that, the inhibition of the growth of Gram-positive bacteria *B. cereus* (MIC = 33.3 μM) is higher than that of Gram-negative *P. aeruginosa* (MIC - 44.7 μM), while in the case of yeasts *C. lipolytica* the activity is lower (MIC = 56.0 μM).

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