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Synthesis and photochromic response of a new precisely functionalized chitosan with "clicked" spiropyran

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ABSTRACT

A new light responsive polysaccharide based on a precisely defined N-phthaloyl chitosan with a covalent bound photochromic spiropyran moiety in C-6 was synthesized through Cu(I) catalyzed azide–alkyne [3+2] dipolar cycloaddition between 6-azido-6-deoxy, N-phthaloyl chitosan and a new spiropyran derivative containing an alkynyl group (SPCC). The reaction product was characterized by NMR and FT-IR spectroscopies for confirming the molecular structure. The UV–vis spectroscopy analyses of films prepared by solution casting on glass from DMF solutions of the functionalized polysaccharide, before and after exposure to an UV lamp (in the 280–350 nm spectral range) or sunlight, showed very slow thermal decay of the photo-induced colored form which was substantially maintained after few months at room temperature. On the contrary, fast color fading was observed for SPCC in ethanol or diethyl ether solution where the color disappeared completely in few minutes at the same temperature. Intermediate color durability was found in diethyl ether suspensions, which indicated the solid polysaccharide phase as responsible of the observed color durability. Considering that the absorption spectra of the polymers are similar under the different conditions examined, the observed effect in the solid state is discussed with reference to the chromophore environment providing molecular and supramolecular hindrance by steric effect and weak binding.

1. Introduction

Chitosan is one of the most abundant biopolymers: it supports countless forms of life, and it is isolated from by-products of the marine food industry. It is a linear polysaccharide of (1–4) linked 2-amino-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-glucose: the acetylation degree is typically less than 0.35 (Jollèsm & Muzzarelli, 1999; Muzzarelli, 2010). Chitosan properties are excellent biodegradability, ecological safety, absence of toxicity, favourable biological activities and chelation capacity for transition metal ions, enabling its applications in materials science and technology (Carreira et al., 2010; Muzzarelli, 2011), pharmaceuticals (Zambito & Di Colo, 2010), biomedicals (Di Martino, Sittinger, & Risbuda, 2005; Muzzarelli & Muzzarelli, 2005), food science (Harish Prashanth & Tharanathan, 2007) and biofabrication (Yi et al., 2005).

Spiropyrans (Fischer & Hirschberg, 1952) are a well known class of organic compounds with the ability to vary their chemical structure when exposed to light of an appropriate wavelength, and regain their original isomer when placed in the dark, or upon exposure to light of a different wavelength (Berkovic, Krongauz, & Weiss, 2000; Pieroni & Ciardelli, 1995). After irradiation, spiropyran converts from closed form (SP) to open colored form, the so-called merocyanine (MC) (Scheme 1). The latter may exist in different geometrical isomers, *cis* and *trans*, the former isomer being unstable and transforming into the latter isomer. Moreover, its electronic distribution can vary from highly zwitterionic (MC-I) to an essentially non-ionic *ortho*-quinoidal (MC-II) structure (Scheme 1) (Lee, Kim, Cho, Lee, & Choi, 2004; Rosario, Gust, Hayes, Springer, & Garcia, 2003).

Merocyanines have also a tendency to associate into stack-like arranged aggregates of *J* and *H* types (Spano, 2010). In *J*-aggregates molecules are parallel and have UV–vis absorption spectrum shifted to the red, as compared with the isolated merocyanine. On the contrary, in *H*-aggregates molecules are antiparallel and the spectrum is shifted to the blue (Berkovic et al., 2000). The interconversion between spiropyran and merocyanine entails chemical and physical variations such as color, dipole moment and optical configuration. If a spiropyran is dispersed or covalently

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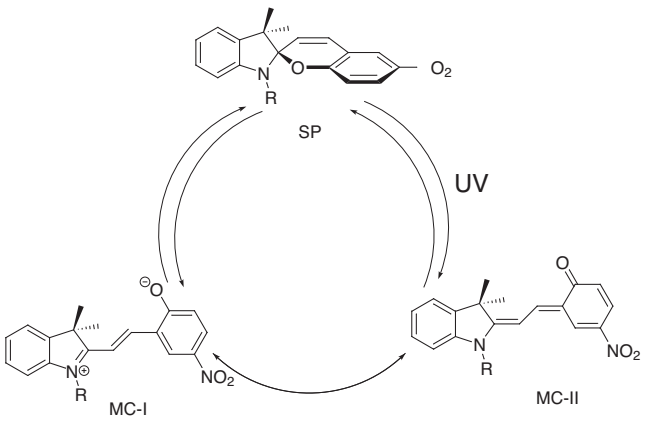


Scheme 1. Closed-ring spiropyran (SP) and corresponding zwitterionic (MR-I) or *ortho*-quinoidal (MR-II) open-ring merocyanine forms.

bound to a polymer it can provide this with several properties such as photochromism, thermochromism (Guglielmetti, 2003), acidochromism (Roxburgh & Sammes, 1995) and electrochromism (Monk, Mortimer, & Rosseinsky, 2007). The covalent binding of spiropyrans into polymeric matrices can significantly reduce their photodegradation that is an important limitation to practical application.

Two are essentially the widespread methods to link spiropyrans to macromolecules: the radical polymerization using vinyl or acrylic spiropyran derivatives as co-monomer (Fu, Sun, Chen, & Yuan, 2008; Miyashita, Nakano, Hirano, & Nohira, 1993), or the post-functionalization of the macromolecule with the dye. The latter is generally performed by the condensation between spiro-compounds containing hydroxyl (Ciardelli, Fabbri, Pieroni, & Fissi, 1989) or carboxyl groups (Edahiro, Sumaru, Takagi, Shinbo, & Kanamori, 2006Fissi, Pieroni, Ruggeri, & Ciardelli, 1995; Hirakura et al., 2004) and complementary functional groups into the macromolecule. Photo-responsive spiro-compounds have been attached to biopolymers from renewable resources such as polypeptide (Ciardelli et al., 1989; Fissi et al., 1993, 1995; Pieroni, Fissi, Viegi, Fabbri, & Ciardelli, 1992), pullulan (Hirakura et al., 2004), methylcellulose (Arai, Shitara, & Ohyama, 1996) and dextran (Edahiro et al., 2006), through condensation, or chitin (Fu et al., 2008) through graft polymerization.

The creation of new materials by combining chromophores and polysaccharides has been recently reviewed by Wondraczek et al. (in press) who pointed out the lack of information about the influence of polysaccharide backbone and regioselectivity of the photo-responsive derivatives on their properties. In this context photo-responsive precisely functionalized chitosan with spiropyran appears of relevant interest. The Cu(I) catalyzed azide–alkyne [3 + 2] dipolar cycloaddition (Finn, Kolb, Fokin, & Sharpless, 2008) (click reaction) has recently become one of the most popular "click" reactions in macromolecular chemistry being extremely regio-selective and occurring under mild conditions (Binder & Sachsenhofer, 2007; Liebert, Hänsch, & Heinze, 2006). To this regard, the high versatility of 6-azido-6-deoxy, N-phthaloyl derivative of chitosan (PH-N_3) as reagent in the "click" reaction with mono- or dialkyne has been recently demonstrated by our group (Zampano, Bertoldo, & Ciardelli, 2010).

In the present paper, a new chitosan derivative was synthesized by regioselectively linking an alkyne functionalized spiropyran (SPCC) to N-phthaloyl chitosan by click reaction. The photochromic response when exposed to UV or sun light of either a film or a dispersion in diethyl ether of the polysaccharide derivative were investigated by UV/vis spectroscopy.

2. Experimental

2.1. Materials

Chitosan (Sigma–Aldrich), average molecular weight 190–210 kDa, degree of deacetylation 0.73 by elemental analysis and ^{1}H NMR spectroscopy, was used as received. 1-Propyn-2-ol (Sigma–Aldrich) was distilled over K_2CO_3 (40 °C, 70 mm Hg) and N,N-dimethylformamide (DMF) from Na_2SO_4 (53 °C, 18 mm Hg). All the other reagents (Sigma–Aldrich) were used without further purification.

2.2. Synthesis of 1-tosyl-2-propyne

1-Propyn-2-ol (22.4 g, 0.40 mol) was added to a diethyl ether *p*-toluenesulfonyl chloride (72.4 g, 0.38 mol, 0.95 equiv.) solution. The reaction mixture was cooled at −10 °C, and an excess of powdered potassium hydroxide (100 g, 4.5 equiv.) was added (20 g at a time over 30–40 min). The reaction was carried at −10 °C for 5 h, quenched with ice water and extracted with diethyl ether. The recovered dried crude product was purified by crystallization (three times) from petroleum ether at −78 °C. Yield: 85%.

TLC (pentane/diethyl ether, 5:1): R_f = 0.25. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS) δ (ppm): 7.79 (d, ^3J (H,H) = 8.5 Hz, 2H; CH aromatic), 7.34 (d, ^3J (H,H) = 8.5 Hz, 2H; CH aromatic), 4.67 (d, ^3J (H,H) = 2.6 Hz, 2H, CH_2), 2.48 (t, ^3J (H,H) = 2.6 Hz, 1H; CH), 2.43 (s, 3H; CH_3).

2.3. Synthesis of 1-propargyl-2,3,3-trimethylindolenine tosylate

2,3.3-Trimethylindolenine (1.1 mL, 7 mmol) and 1-tosyl-2-propyne (1.5 g, 7 mmol) were heated under nitrogen at 70 °C for 2 h. The resulting solid material was dissolved in CH_2Cl_2 (10 mL) and filtered through silica gel (CH_2Cl_2, then methanol). The solid recovered by solvent evaporation was partially purified by crystallization from CH_2Cl_2/hexane. Yield: 70%.

^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 7.77–7.10 (m, 8H; CH aromatics), 5.63 (s, 2H; CH_2), 3.09 (s, 3H; CH_3), 2.64 (s, 1H; CH), 2.35 (s, 3H; TS-CH_3), 1.65 (s, 6H; CH_3).

2.4. Synthesis of 1′,3′-dihydro-3′,3′-dimethyl-1′-propargyl-6-nitrospiro[2H-1-benzopyran-2,2′-(2H)-indole] (SPCC)

1-(2′-Propynyl)-2,3,3-trimethylindolenine tosylate (1.2 g, 3 mmol) was dissolved in 15 mL of 2-butanone. 5-Nitrosalicylaldheide (0.53 g. 3 mmol) and piperidine (0.32 mL, 3 mmol) were added, and the reaction mixture was stirred for 3 h at 90 °C and then 15 h at room temperature. The crude product in CH_2Cl_2 solution was filtered through silica gel. Yield: 48%.

Elemental analysis: found C72.53. H5.74, N7.89. TLC ($CHCl_3$): R_f = 0.88. IR (KBr, Fig. 2): 3282 (ν, ≡CH), 3051 (ν, Ar–H), 2965 (ν, CH_3), 2927 (ν, CH_3), 2868 (ν, CH_2), 2116 (ν, C≡C), 1610 (ν, C=C), 1518 cm^{-1} (NO_2), 1481 (δ, aliphatic C–H), 730 cm^{-1} (δ, aromatic C–H). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS, Fig. 1): δ = 8.02 (dd, ^3J (H_4,H_3) = 9.88 Hz, 1H, ^3J (H_4,H_5) = 2.62 Hz; H_4), 8.01 (d, ^3J (H_5,H_4) = 2.62 Hz, 1H; H_5), 7.24 (dd, ^3J (H_{11},H_{12}) = 7.86 Hz, ^3J (H_{11},H_{10}) = 7.45 Hz, ^3J (H_{11},H_9) = 1.21 Hz, 1H; H_{11}), 7.12 (dd, ^3J (H_9,H_{10}) = 7.25 Hz, ^3J (H_9,H_{11}) = 1.21 Hz, 1H; H_9,), 6.97 (d, 1H, ^3J (H_7,H_6) 10.36 Hz; H_7), 6.94 (dt, ^3J (H_{10},H_{11}) = 7.46 Hz, ^3J (H_{10},H_9) = 7.25 Hz, ^3J (H_{10},H_{12}) < 1 Hz, 1H; H_{10}), 6.65 (d, ^3J (H_3,H_4) = 9.88 Hz, 1H; H_3,), 5.89 (d, ^3J (H_6,H_7) = 10.36 Hz, 1H; H_6,), 4.06 (dd, ^3J (H_2,$H_{2'}$) = 18.0, ^3J (H_2,H_1) = 2.64 Hz, 1H; H_2), 3.85 (dd, ^3J ($H_{2'}$,H_2) = 18.0, ^3J ($H_{2'}$,H_1) = 2.44 Hz, 1H; $H_{2'}$), 2.10 (dd, ^3J (H_1,H_2) = 2.64 Hz, ^3J (H_1,$H_{2'}$) = 2.44 Hz, 1H; H_1), 1.31 (s, 3H; H_8), 1.20 (s, 3H; $H_{8'}$). ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C, TMS): δ = 159.5, 145.9,

141.3, 136.2, 129.1, 128.0, 126.2, 123.0, 122.0, 121.2, 120.7, 118.5, 116.0, 108.3, 106.1, 79.9, 71.8, 52.8, 32.8, 26.2, 20.1.

2.5. Synthesis of N_3-functionalyzed-N-phthaloyl-chitosan (PH-N_3)

The chitosan modification to N_3-functionalyzed-*N*-phthaloyl-chitosan was carried out in two reaction steps, as reported by Zampano et al. (2010). Briefly, in the first reaction step chitosan (5.0 g, 31 mmol of monomer) was reacted with phthalic anhydride (11.5 g, 78 mmol) in DMF (100 mL) at 130 °C under nitrogen atmosphere for 6 h. The mixture was then precipitated in ice-water, collected by filtration, washed by Soxhlet extraction with ethanol for 8 h and dried under vacuum for 24 h. Elemental analysis: found C55.87, H4.52, N3.75. The degree of substitution with phthaloyl groups (DS_{Ph} = 1.35) was estimated by the increase in the molar C/N ratio between chitosan (C/N = 6.54) and N-phthaloyl-chitosan (C/N = 17,36). IR (KBr, Fig. 2): 3450 (ν, OH), 1776 (ν_a, C=O), 1712 (ν_s, C=O), 1290–1260 (C–O ester), 1150–1000 (pyranose ring), 721 (δ, aromatic ring). ^{1}H NMR (300 MHz, [D_6]DMSO, 25 °C, TMS): δ = 1.7 (CH_3 acetamide), 3.4–5 (pyranose ring), 7.2–7.9 (phthaloyl). ^{13}C NMR (75 MHz, [D_6]DMSO, TMS): δ = 57.3, 60.4, 72.3, 75.4, 80.2, 101.8, 98.3, 123.9, 128.9, 131.7, 133.4, 135.1, 167.8. In the second reaction step N-phthaloyl-chitosan (5 g, 14.33 mmol of monomer), carbon tetrabromide (11.9 g, 35.8 mmol) and triphenylphosphine (9.40 g, 35.8 mmol) were stirred in DMF (250 mL) for 24 h at 80 °C. Sodium azide (9.32 g, 143.3 mmol) was added and stirred for 24 h thus the polymer was precipitated first in ethanol and then in toluene. The product was collected by filtration, washed with toluene, ethanol, diethyl ether and dried under vacuum. Elemental analysis: found C48.16, H4.27, N8.30. The degree of azide functionalization per monomeric unit (DS_{N3}) was 0.28 from FT-IR and ^{1}H NMR spectroscopies analyses. IR (KBr, Fig. 2): 3450 (ν_s, OH), 2110 (ν, N_3), 1776(ν_a, C=O), 1712 (ν_s, C=O), 1150–1000 (pyranose ring), 721 cm^{-1} (δ, aromatic ring). ^{1}H NMR (300 MHz, [D_6]DMSO, 25 °C, TMS): δ = 1.7 (CH_3 acetamide), 3.4–5.0 (pyranose ring), 7.8 (phthaloyl). ^{13}C NMR (75 MHz, [D_6]DMSO, 25 °C, TMS): δ = 50.1, 57.3, 60.4, 69.4, 72.3, 75.4, 80.2, 98.3, 101.8, 123.9, 131.7, 135.1, 167.8.

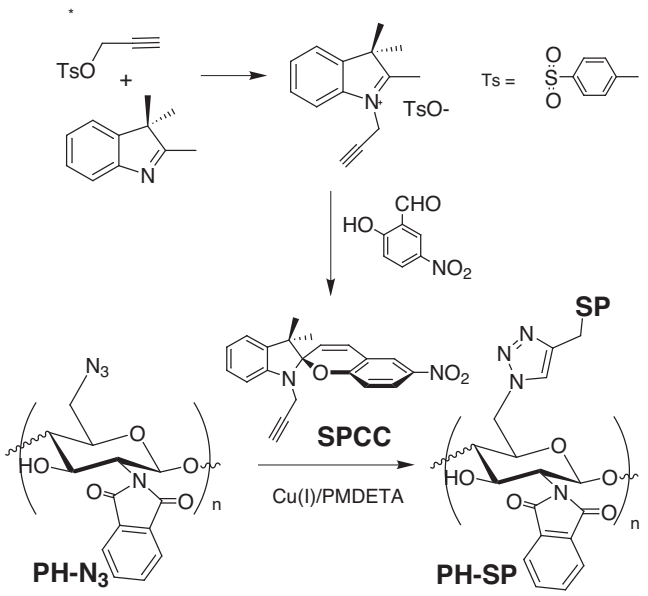
2.6. Synthesis of spiropyran-functionalized-N-phthaloyl-chitosan (PH-SP)

1′,3′-Dihydro-3′,3′-dimethyl-1′-propargyl-6-nitrospiro[2H-1-benzopyran-2,2′-(2H)-indole] (SPCC) (0.195 g, 1.1 equiv.) and 0.03 mL of an aqueous, freshly prepared, 4 M solution of sodium ascorbate (0.25 equiv.) were added to a 1.4% (w/v) solution of PH-N_3 in DMF. CuBr (0.037 g, 0.5 equiv.) with N,N,N′,N″,N″-pentamethyldiethylenetriamine (PMDETA) (0.05 mL, 0.5 equiv.) was used as catalyst. The reaction mixture was stirred at 40 °C for 60 h under nitrogen, then precipitated twice (diethyl ether and acetone), filtered, washed with water, acetone, diethyl ether and dried under vacuum.

IR (KBr, Fig. 2): 3450 (νs, OH), 1776 (ν_a, C=O), 1712 (ν_s, C=O), 1150–1000 (pyranose ring), 721 cm^{-1} (δ, aromatic ring). ^{13}C NMR (75 MHz, [D_6]DMSO, 25 °C, TMS, Fig. 3): δ = 19.2 (C-i), 25.3 (C-j), 51.9 (C-h), 56.5 (C-2), 59.3 (C-6′), 68.3 (C-6), 72.3 (C-3), 74.7 (C-5), 79.1 (C-4), 96.5 (C-1), 105.9 (C-m), 106.9 (C-k), 115–146 (aromatic), 158.5 (C-s), 169.2 (C-7).

2.7. Instruments and methods

^{1}H and ^{13}C NMR measurements were performed on a NMR spectrometer operating at 300 MHz and 75 MHz, respectively. ^{1}H and ^{13}C NMR chemical shifts were referred to TMS as external standard. FT-IR spectra were recorded with the help of a Perkin-Elmer Spectrum One spectrometer on KBr dispersion. UV–vis absorption spectra were recorded with Perkin-Elmer Lambda 650, spectrophotometer. Data were analyzed with Origin 7.5, software by Microcal Origin®. The scattering contribution was corrected by appropriate baselines. Elemental analyses were accomplished by the microanalysis laboratory at the Faculty of Pharmacy, University of Pisa.



Scheme 2. Synthesis of SPCC and PH-SP.

The concentration of SPCC solutions in diethyl ether and ethanol for UV measurements was 10^{-5} M. PH-SP and PH-N_3 suspensions were obtained from their DMF solutions (20 g L^{-1}) by diluting 1/60 with diethyl ether. PH-SP films were prepared by solution casting on glass surface from a 20 g L^{-1} solution in dry DMF. Irradiation of samples was performed under sunlight (570 (W cm^{-2}) or UV lamp (125 W, Hg vapor, water filter, distance 25 cm) for 1–5 min. Moreover, irradiation with green light was performed with a laser operating at 540 nm, 85 mW, for 3–10 min at a distance of 20–25 cm from the film.

3. Results and discussion

The 1,3,3-trimethyl-2-methylene indoline (Fischer's base) is the common building block to form N-methyl-spiropyrans. However, in the case of a differently substituted spiropyrans, the required indoline has to be synthesized from 2,3,3-trimethylindolenine and the desired electrophilic compound prior to the condensation with salicylaldehyde (Lukyanov & Lukyanova, 2005). Thus, the spiropyran containing an alkyne moiety (SPCC) was synthesized by us in three steps (Scheme 2). First, 1-propyn-3-ol was activated by tosylation for the addition to the nitrogen of 2,3,3-trimethylindolenine in the second reaction step that gave 1-propargyl-2,3,3-trimethylindolenine tosylate. This last resulted scarcely stable in solution and can be stored only in solid state, thus it was hurriedly purified and used. Finally, the condensation with 5-nitrosalicylaldheide in the presence of piperidine gave 1′,3′-dihydro-3′,3′-dimethyl-1′-propargyl-6-nitrospiro[2H-1-benzopyran-2,2′-(2H)-indole] (SPCC).

The ^{1}H NMR spectrum of SPCC (Fig. 1) showed the proton resonance of terminal alkyne at 2.10 ppm and of α-methylenic group (H_2, $H_{2'}$) at 4.06 ppm and 3.85 ppm. The chemical shifts and the nonequivalence of diastereotopic methylenic protons indicate the binding of propyne moiety to nitrogen through a covalent bond. All the other proton resonances appear in the exact intensities and

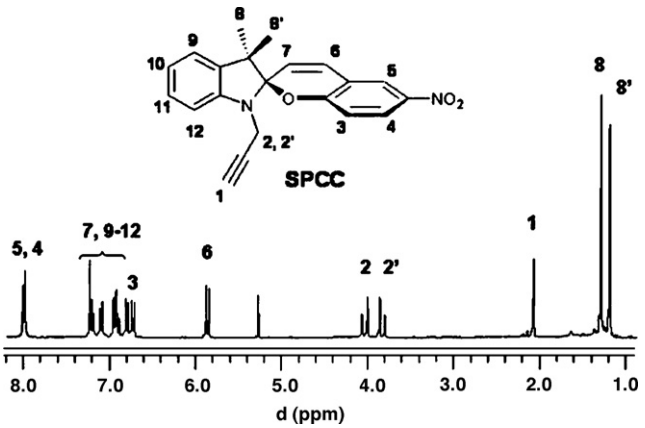


Fig. 1. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, 20 mM) of SPCC.

are correlated to commercial available spiropyran (Sigma–Aldrich), thus confirming the expected molecular structure of SPCC. The attribution of the ^{1}H NMR signals is reported in Fig. 1.

The absence of detectable unassigned signals indicates that SPCC was obtained with purity suitable for the following reaction. FT-IR (Fig. 2), ^{13}C NMR spectroscopies and elemental analysis further confirmed the structure of SPCC and the absence of detectable impurities.

The behavior of functionalized chitosan can be affected from the position of the link with the derivatizing agent. Indeed, the derivatization in C-6 position, rather than C-2 or C-3, lead to less rigid network because of its larger conformational freedom. Therefore, to avoid high chitosan fragility, we synthesized 6-azido-6-deoxy, N-phthaloyl chitosan (PH-N_3), as selective reagent for C-6 linking SPCC to the polysaccharide. In the first reaction step the primary amine groups of chitosan were converted to N-phthaloyl groups, by reacting chitosan with phthalic anhydride under conventional conditions (Nishimura, Kohgo, Kurita, & Kuzuhara, 1991). Then the primary –OH groups in C-6 were modified in one-pot reaction, namely bromination and substitution with azide. The product was effectively functionalized with N_3 groups as confirmed by the presence of the azide stretching band at 2110 cm^{-1} in the IR spectrum (Fig. 2). Moreover the ^{1}H and ^{13}C NMR spectra were in accordance to the already reported ones (Zampano et al., 2010). The degree

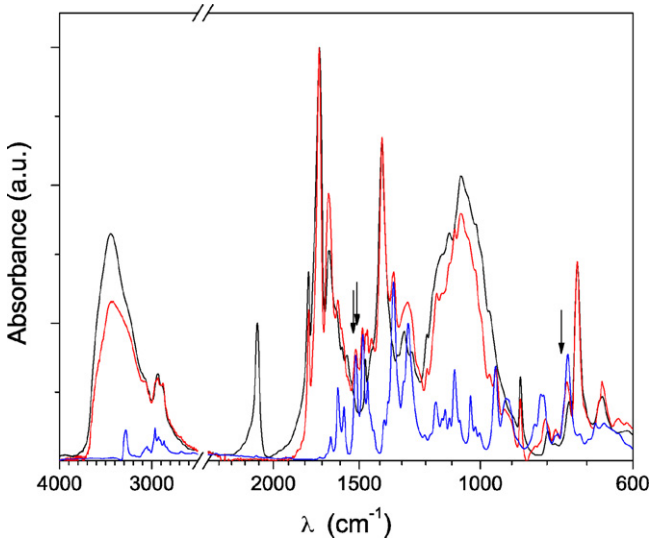


Fig. 2. FT-IR (KBr) spectra of SPCC (blue), PH-N_3 (black) and PH-SP (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of substitution with –N_3 groups per pyranose ring (DS-N_3) was 0.28, as estimated by elemental analysis. Thus, in the third reaction step the functionalized chitosan was simply modified with SPCC under "click" conditions to quantitatively effort 6-spiropyran-N-phthaloyl chitosan (PH-SP, DS spiropyran ~0.25) (Scheme 2). PH-SP showed better solubility in the common aprotic polar solvents (DMSO, DMF, NMP) than PH-N_3 most likely due to the less ordered structure and the higher hydrophobic character of the prepared derivative than PH-N_3.

The ^{13}C NMR spectrum of PH-SP (Fig. 3) showed signals due to both the N-phthaloyl chitosan and the spiropyran moiety: the resonances at 19.2 ppm and 25.3 ppm were assigned to the methyl groups of spiropyran, coded C-i and C-j (Fig. 3), according to the SPCC ^{13}C NMR spectrum. Quaternary carbon of indoline portion of SP function, coded C-h, resonated at 51.9 ppm. The aliphatic region of the spectrum between 56 ppm and 80 ppm belong to the pyranose ring of the carbohydrate chain, and the signal at 96.5 ppm is produced by the anomeric carbon C-1, according to literature data (Satoh et al., 2006). It is noteworthy that the signal at 50.1 ppm, which was attributed to the resonance of the C-6 linked with the –N_3 moiety (Satoh et al., 2006; Zampano et al., 2010) was not detectable, indicating the disappearing of the azide moiety. The olefinic carbon linked to the nitro-aromatic ring and the quaternary carbon bound to both the nitrogen and oxygen atoms resonated at 105.9 ppm and 106.9 ppm. An extended aromatic region formed by superimposed signals is observed from 115 ppm to 146 ppm. The three well intense resonances at 122.9 ppm, 131.2 ppm and 134.3 ppm were assigned to the N-phthaloyl aromatic ring, while the remaining superimposed signals belong to aromatic moiety of spiropyran. Based on literature (Marra, Vecchi, Chiappe, Melai, & Dondoni, 2008), the low intense signal at 142.5 ppm, which was not observed neither in SPCC nor in PH-N_3 spectra, was attributed to the quaternary carbon of the formed triazole group. Furthermore, the two signals at high frequencies (158.5 ppm and 169.2 ppm) are produced by the quaternary carbon of the condensed aromatic ring with –NO_2 group linked to the oxygen atom (C-s) and the C=O of the N-phthaloyl moiety (C-7), respectively. The effective functionalization of N-phthaloyl chitosan with SP groups was further confirmed by the presence of the infrared absorption bands of SPCC at 1518 cm^{-1}, 1481 cm^{-1} and 730 cm^{-1} in the IR spectrum of PH-SP. The comparison of Fig. 2 clearly indicates that these bands are due to SPCC moieties. Similarly, the infrared spectrum showed the band at 1712 cm^{-1} due to the carbonyl stretching of the phthaloyl groups and the complex band at 1000–1150 cm^{-1} due to the pyranose ring, which indicates the maintenance of the N-phthaloyl-chitosan molecular structure. Finally, the disappearing of the typical azide stretching band at 2110 cm^{-1} in the PH-SP IR spectrum confirmed the SPCC bonding through 1,3-dipolar cycloaddition (Fig. 2). Indeed, a blank experiment on N-phthaloyl-chitosan showed that "click" condition did not cause any change in the PH-N_3 structure and spectrum.

3.1. SPCC photochromism

SPCC in diethyl ether (Et_2O) solution showed a dramatic color change from a pale yellow to intense blue after 1 min of UV or sunlight irradiation, due to the isomerization of spiropyran (SP) to merocyanine (MC) form via the photochemical cleavage of the spiro C–O bond (Scheme 1) (Berkovic et al., 2000). The blue color quickly bleached under the room light at room temperature. The UV–vis spectrum showed a band with a maximum at 607 nm due to the MC form (Fig. 4). Indeed, the π-electrons of the two aromatic rings do not interact reciprocally in the SP form, and the adsorption spectrum is the sum of two parts of the molecule. On the contrary, the extended π-electron delocalization of the highly conjugated structure of MC form provides absorption band in the visible region.

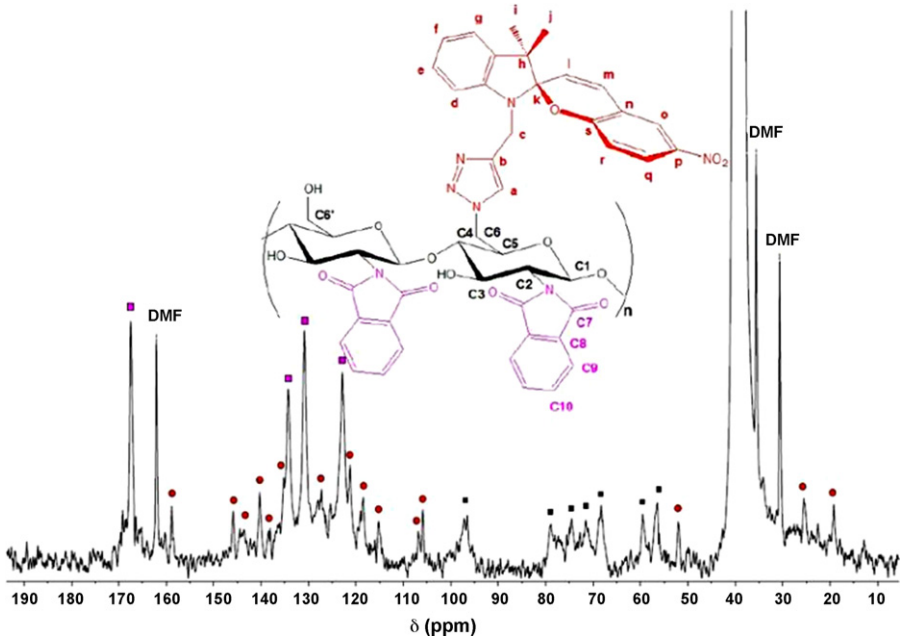


Fig. 3. ^{13}C NMR (75 MHz, [D6]DMSO, 25 °C, 60 mg mL^{-1}) of PH-SP.

SPCC in ethanol solution turned to pink upon UV irradiation with maximum absorption band in the UV–vis spectrum at 564 nm. The explanation of the observed solvent-dependent photochromism, for which the absorption band at 607 nm in apolar solvent shifts to lower wavelength in polar ones, has been matter of controversy. In fact some authors ascribed the band at 607 nm to quinoidal MC-II form, while the band at 564 nm to zwitterionic MC-I form (Fig. 4) (Lee et al., 2004; Rosario et al., 2003). Someone else invokes the formation of MC aggregates in apolar solvents (Barachevskii & Karpov, 2007). Indeed, the zwitterionic MC-I form cannot be stable in apolar environment and can be stabilized by forming couples were the negative charge of one molecule is stabilized by the positive charge of the other (Barachevskii & Karpov, 2007).

As expected, the stability of the colored form was higher in polar solvents than in apolar ones such as the spectrum could be collected at room temperature in ethanol, while only at −77 °C in diethyl ether.

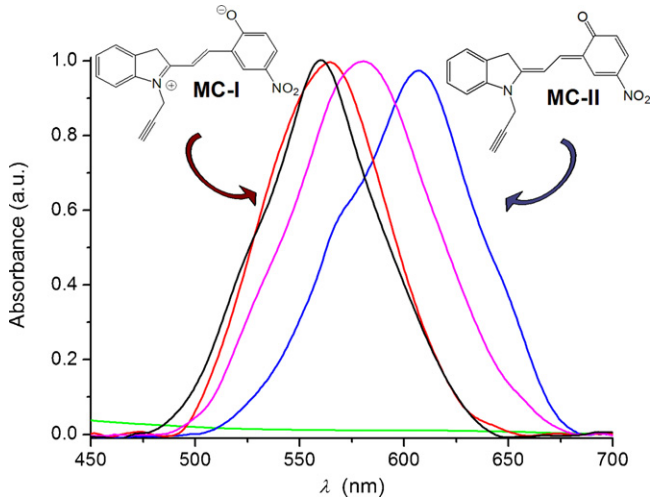


Fig. 4. UV–vis absorption spectra of SPCC diethyl ether solution before (green) and after irradiation at −77 °C (blue), irradiated SPCC ethanol solution (red), irradiated PH-SP diethyl ether suspension (pink), irradiated PH-SP film (black). MC-I (zwitterionic) and MC-II (*ortho*-quinoidal) forms of merocyanine. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. Spiropyran-functionalized-N-Phthaloyl-chitosan (PH-SP) photochromism

Suspensions of PH-SP (∼ 0.5 mg mL^{-1}), PH-N_3 (∼ 0.5 mg mL^{-1}) containing SPCC (0.5 mM) and reference solution of SPCC (0.5 mM) in Et_2O were exposed to sunlight, turning colored. SPCC and PH-N_3/SPCC showed an intense blue color, whereas PH-SP suspension was pink/violet (Fig. 5a and b). This is consistent with the effective SPCC binding to chitosan in PH-SP, which keeps it close to the polar environment provided by the polysaccharide. On the contrary, the unbound SPCC in the mixture suspension is blue, like SPCC in absence of the polysaccharide. Actually, the pink-violet color of the PH-SP suspension, which corresponds to a vis absorption band with maximum at 580 nm (Fig. 4), indicates the presence of both the two different MC forms. As previously discussed, several authors have proposed that the solvatochromic behavior of merocyanines is due to the formation of *J*- or *H*-aggregates (Ando, Miyazaki, Morimoto, Nakahara, & Fukuda, 1985; Barachevskii & Karpov, 2007). However, these aggregates generally show large vis absorption bands with a superimposed sharp peak at wavelength higher than 600 nm. The spectra we observed, did not show such kind of shape. Thus, we interpreted the polarity-induced chromism variation in PH-SP/Et_2O system as the superimposition of zwitterionic MC-I and *ortho*-quinoidal MC-II forms (Lee et al., 2004; Patel et al., 2010; Rosario et al., 2003). The former is due to moieties surrounded by the polysaccharide into the core of the solid particles (polar environment) while the latter to moieties located in the particle shell exposed to the apolar solvent.

The PH-SP suspension was still intensely pink-violet 1 h after from the UV exposure, while sample with unbound SPCC bleached in few minutes in the presence or absence of suspended PH-N_3 (Fig. 5c). Furthermore, the PH-SP suspension color was still evident after 24 h at room temperature. Consistently, the MC absorption band intensity of PH-SP suspension progressively decreased overtime but still maintained ∼1/10 of its initial value after 24 h (Fig. 6).

The maximum of the absorption band of the system PH-SP/Et_2O progressively shifted toward blue during aging in the dark (Fig. 6).

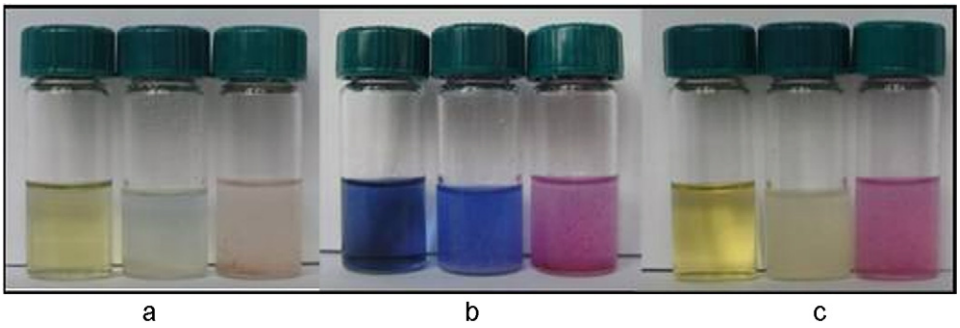


Fig. 5. Pictures relative to SPCC solution in diethyl ether (left side vial), PH-N_3 + SPCC mixture in diethyl ether (central vial) and PH-SP suspended in diethyl ether (right side vial), (a) before, (b) immediately after and (c) after 1 h from UV irradiation.

Thus, a faster decay of the band contribution due to MC-II than MC-I is indicated and this corresponds to higher thermal stability of the zwitterionic form (Fig. 6).

The enhanced life-time of merocyanine in PH-SP can be explained by the polysaccharide inducing polar environment (Adelmann, Mela, Gallyamov, Keul, & Moeller, 2009; Liu, Jiang, Liang, & Gao, 2005), which stabilizes more MC-I form than MC-II (Kim, Oh, Kim, Cha, & Kang, 2008; Nakao, Noda, Horii, & Abe, 2002; Smets, 1983). Furthermore, the chemical linkage between SPCC and the polymer (Smets et al., 1983) limits the conformational mobility of the corresponding merocyanine which cannot easily rotate and isomerize. Indeed, the *trans–cis* isomerization is the rate determining step for the merocyanine ring closure to give back the spiropyran (Biteau, Chaput, & Boilot, 1996). The low free volume of the polymer in the glassy state further reduces the merocyanine mobility, while less rigidity can cause a faster kinetic constant of the decoloration (Kim et al., 2008).

PH-SP film prepared by casting DMF solution on a glass surface appeared pale yellow if kept dry in the dark and turned to pink/violet after exposure to sunlight or UV irradiation (Fig. 7). The UV–vis spectrum of the solid film after irradiation showed an absorption band with maximum at 563 nm tentatively attributed to MC-I form. This value is similar to the value measured in the case of SPCC in ethanol solution (Fig. 4), thus confirming the MC-I stability in polar environment. Surprisingly, the color did not bleach, at least after two months in the laboratory conditions. Actually, the merocyanine band intensity in the film was followed over 100 h (Fig. 7): after an initial small decrease the observed variation was

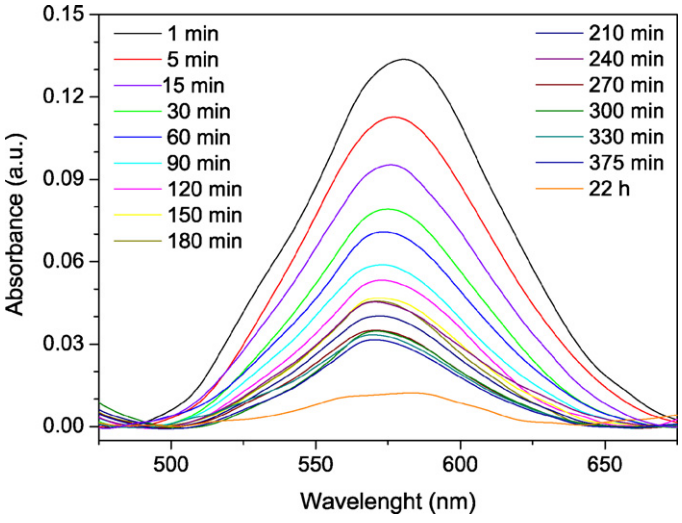


Fig. 6. Overlay UV–vis spectra illustrating the decay of MC to SP form at 25 °C of PH-SP in ether dispersion.

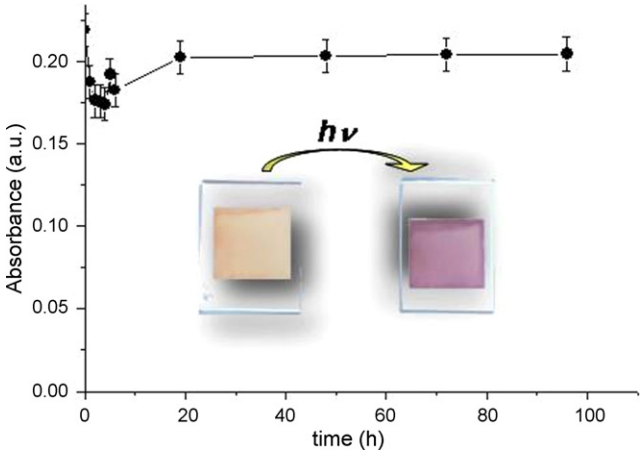


Fig. 7. Adsorption intensity at 563 nm in the UV–vis spectrum of PH-SP film cast on a glass surface at different times from UV irradiation.

comparable to the standard deviation of data thus confirming the merocyanine durability, provided by the polysaccharide environment. Preliminary study proved the process reversibility at least for few cycles: the film violet/pink colored after exposure to sunlight, decolored by irradiation with green laser and turned violet/pink again after further exposure to sunlight. It could be inferred that the high linear and stereo ordered structure of chitosan that results in closed packed parallel chains and strong intermolecular electrostatic interactions contributes to the MC-I form stabilization by slowing down the *trans–cis* isomerization (Kinashi, Harada, & Ueda, 2008). Furthermore, possible interaction between the nitrogen atom of chitosan and the zwitterionic merocyanine can result in interchain bonding and thus rigidity increase of the functionalized polymer (Kim et al., 2008). Even if the protection of the amine groups of chitosan may reduce this interaction, bulky aromatic substituents in the vicinity of spiro-moieties in polymer matrices can affect the decoloration rate (Nakao et al., 2002), pointing out the positive effect of N-phthaloyl group in the stabilization of the MC form with respect to free amine groups (Fu et al., 2008).

4. Conclusions

We implemented a selective and precise synthetic strategy to obtain N-phthaloyl chitosan regioselectively functionalized in C-6 position with a photochromic moiety. Copper catalyzed alkyne–azide [3 + 2] dipolar cycloaddition (click) was exploited as selective synthetic approach. In particular, a new spiropyran derivative with propyne pedant group was synthesized here for the first time and reacted with azide C-6 functionalized N-Phthaloyl chitosan.

The new chitosan based material with covalently bound spiropyran groups showed an interesting photochromic response. Indeed, after UV light irradiation, the colorless material became intensely colored either in Et_2O suspension or solid film due to the conversion of the spiropyran in the merocyanine form. The photo-excited merocyanine form lived more than 24 h as suspension in diethyl ether and more than two months as dry film at room temperature. By contrast, the unbound spiropyran in ethanol or diethyl ether bleached in few minutes. This very low thermal recovery of the photo-generated open merocyanine form in the solid state can be ascribed to chemical and physical stabilization of the open merocyanine by mainly nonbonded environmental effects derived by the embedment of the chromophore into the chitosan material. These findings indicate a very well controlled route to provide a polysaccharide, in particular chitosan, with a specific photoresponse to light irradiation which is affected and modified to large extent by chromophore–biomacromolecule nonbonded interaction.

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