INTECH OPEN ACCESS PUBLISHER

Photoresponsive Cellulose Nanocrystals

Regular Paper

Ilari Filpponen[3], Hasan Sadeghifar[1] and Dimitris S Argyropoulos[1,2*]

1 Organic Chemistry of Wood Components Laboratory, Department of Forest Biomaterials, North Carolina State University, United States
2 Laboratory of Organic Chemistry, Department of Chemistry, Faculty of Science, University of Helsinki, Finland
3 Department of Forest Products Technology, School of Chemical Technology, Aalto University, Finland
*Corresponding author E-mail: dsargyro@ncsu.edu

Received 26 May, 2011; Accepted 20 July, 2011

Abstract In this communication a method for the creation of fluorescent cellulose nanoparticles using click chemistry and subsequent photodimerization of the installed side-chains is demonstrated. In the first step, the primary hydroxyl groups on the surface of the CNCs were converted to carboxylic acids by using TEMPO-mediated hypohalite oxidation. The alkyne groups, essential for the click reaction, were introduced into the surface of TEMPO-oxidized CNCs via carbodiimide-mediated formation of an amide linkage between monomers carrying an amine functionality and carboxylic acid groups on the surface of the TEMPO-oxidized CNCs. Finally, the reaction of surface-modified TEMPO-oxidized cellulose nanocrystals and azido-bearing coumarin and anthracene monomers were carried out by means of a click chemistry, i.e., Copper(I)-catalyzed Azide-Alkyne Cycloaddition (CuAAC) to produce highly photo-responsive and fluorescent cellulose nanoparticles. Most significantly, the installed coumarin and/or anthracene side-chains were shown to undergo UV-induced [2+2] and [4+4] cycloaddition reactions, bringing and locking the cellulose nanocrystals together. This effort paves the way towards creating, cellulosic photo responsive nano-arrays with the potential of photo reversibility since these reactions are known to be reversible at varying wavelengths.

Keywords cellulose, click chemistry, fluorescence, photodimerization

1. Introduction

Cellulose is the most abundant and low-cost natural polymer. By virtue of its non-toxicity, biocompatibility and degradability cellulose has broad applications in the food, pharmaceutical and chemical industries. However, economically feasible and environmentally friendly chemical processes are required for more efficient utilization of cellulose [1]. Therefore, numerous efforts are underway seeking new ways to expand the application range of cellulose while retaining its prominent properties. For example, in the field of photo-luminescence, the cellulose needs to be chemically modified to achieve desirable optical properties [2]. Typically, this is accomplished by covalently attaching fluorescent dyes on the surface of cellulose either directly or by using cross-linking agents [3, 4].

Cellulose and cellulose derivatives offer several advantages as backbones for the creation of photo-responsive polymers. The presence of optically active centers is of particular interest with respect to the formation of a cholesteric state of liquid crystalline properties and the associated interactions with chromophoric molecules [5-8]. Cellulose derivatives that are soluble in some solvents can be used for the preparation of high quality thin films [9, 10]. Thus, if these photoresponsive molecules can be introduced into

cellulose and cellulose derivatives, these cellulose and cellulose derivatives could be novel materials for photoresponsive polymers. Furthermore, they might be used as photoreactive materials for applications such as photorecording devices, liquid crystal displays, and other light-sensitive applications.

Efforts have been made to make photoresponsive cellulose derivatives. To date, there are several papers that attempt to prepare photoresponsive cellulosic polymers with the goal of preparing new materials with the enumerated advantages of the starting substrate. Most photoresponsive molecules introduced into cellulose derivatives are azobenzene, cinnamate and stilbene molecules [11-15]. The cellulose derivatives containing azobenzene were found to show photoreactivity by UV light irradiation and to form lyotropic liquid crystalline phase [16, 17]. However, there are very few trials on cellulose or other biopolymer derivatives containing coumarin or anthracene molecules, although they can be expected to possess photoreactivity and also to form a liquid crystalline phase [18-20].

The photochemistry of anthracene and its derivatives has been widely studied [3, 21-24]. Anthracene has four distinct UV absorption bands in the wavelength range of 275-325 nm and an irradiation by wavelengths higher than 350 nm induces a ($4\pi + 4\pi$) cycloaddition reaction leading to the formation of a photodimer. Moreover, the photodimers do not absorb light of wavelengths >300 nm due to the reduction of the conjugated system (from anthracene to ortho-disubstituted benzene) by the photo-addition process. Such a shift between the absorption spectra is an advantage for the photochromic properties of the system. Moreover, in the wavelength range of 250-290 nm anthracene photodimers are known to photodissociate into two anthracene nuclei [25, 26]. The applications of anthracene in fluorescent sensors [27-30], dendrimeric compounds [31] and copolymers [32, 33] have been reported by several research groups.

In this paper, we report the synthesis of fluorescent cellulose nanocrystals (CNCs) by covalently linking of fluorescence chromophores to the cellulose surface. First, the primary hydroxyl groups on the surface of the CNCs were converted to carboxylic acids by using TEMPO-mediated hypohalite oxidation. In the next step, a compound (propargylamine) carrying a terminal amine functionality was grafted on to the oxidized CNCs via carbodiimide-mediated formation of an amide linkage between an amine and the carboxylic groups on the CNC's surface. The grafted amine also contained an alkyne functionality which was then used as the reaction site for further modification. These modified CNCs were finally subjected to Click chemistry reaction conditons [34] i.e. Copper(I)-catalyzed Azide-Alkyne Cycloaddition (CuAAC) with an azide-bearing fluorescent coumarin and anthracene chromophores resulting in highly fluorescent nano cellulosic materials. Finally, the produced materials were subjected to photochemical irradiation inducing [2+2] and [4+4] cycloaddition reactions respectively between the installed coumarin and anthracene side chains, causing the photo-induced formation of cellulosic based crystalline nano-arrays. Current efforts to reverse these reactions at lower wavelengths will pave the way toward the creation of photo induced tunable cellulosic based nanomaterials.

2. Experimental Details

Whatman #1 filter paper was used as a starting material for the cellulose nanocrystals. Propargylamine (98%) was purchased from Aldrich. All other chemicals were purchased from Aldrich or Fisher and used as received unless otherwise stated.

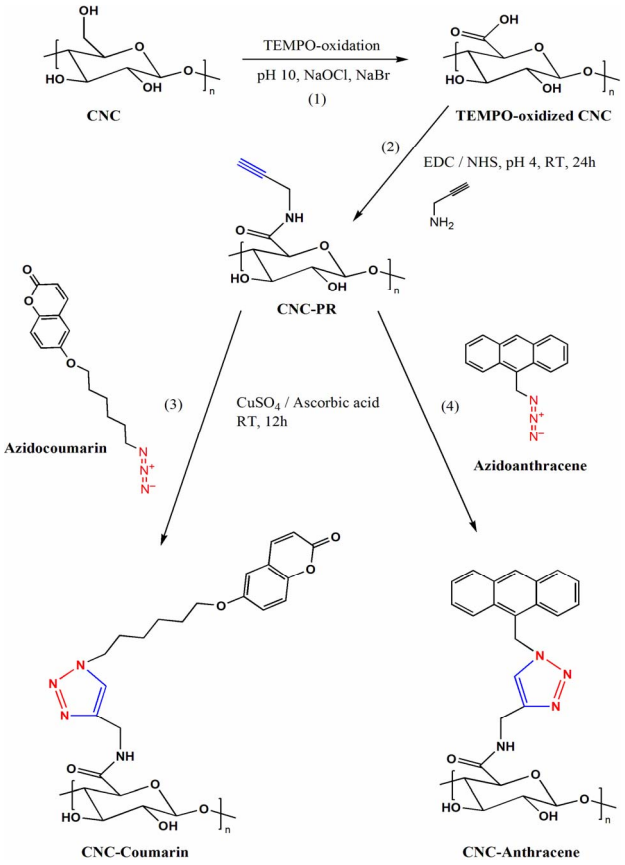
2.1 Acid Hydrolysis of Cellulose to Produce Cellulose Nanocrystals (CNC)

The cellulose nanocrystals were formed by acidic hydrolysis similar to the procedure used by Araki *et al* [35-37]. A typical procedure was as follows. 2.0 g of cellulose pulp obtained from Whatman #1 filter paper (98% α-cellulose, 80% crystallinity) was blended by a 10 Speed Osterizer® Blender. Resulting pulp was hydrolyzed with 100 mL of 2.5 M HBr at 100°C for 3 hours. The ultrasonication was applied during (every 60 minutes) the reaction (Omni-Ruptor 250W ultrasonic homogenizer, 50% power, 5 min). The resulting mixture was diluted with de-ionized (D.I) water followed by five cycles of centrifugation at 1500g for 10 min. (IEC Centra-CL3 Series) to remove excess acid and water soluble fragments. The fine cellulose particles became dispersed in the aqueous solution approximately at pH 4. The turbid supernatant containing the polydisperse cellulose particles was then collected for further centrifugation at 15000 g for 45 min (Automatic Servall® Superspeed Centrifuge) to remove ultra-fine particles. Ultra-fine particles with small aspect ratio were removed from the upper layer, and the precipitation (after the high-speed centrifugation) was dried using a lyophilizing system (Labconco, Kansas City, MU).

2.2 TEMPO-mediated Oxidation of CNCs

648 mg (4 mmol of glucosyl units) of cellulose nanocrystals were suspended in water (50 mL) containing 10 mg of 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO, 0.065 mmol) and 200 mg of sodium bromide (1.9 mmol) at room temperature for 30 min. The TEMPO-mediated oxidation of the cellulose nanocrystals was initiated by slowly adding 4.90 mL of 13% NaClO (8.6 mmol) over 20

min at room temperature under gentle agitation. The reaction pH was monitored using a pH meter and maintained at 10 by incrementally adding 0.5 M NaOH. When no more decrease in pH was observed, the reaction was considered complete. About 5 mL of methanol was then added to react and quench with the extra oxidant. After adjusting the pH to 7 by adding 0.5 M HCl, the TEMPO-oxidized product was washed with D.I. water by centrifugation and further purified by dialysis against D.I. water for two days. 550 mg of solid was recovered after freeze-drying (see Scheme 1, step 1). FTIR measurements showed a carboxylic acid peak at 1730 cm^{-1}.



Scheme 1. Schematic representation of the synthetic route for the photoresponsive cellulose nanocrystals.

2.3 Preparation of 6-((6-hydroxyhexyl)oxy)coumarin

A solution of 6-bromohexanol (2g, 12.3mmol), 6-hydroxycoumarin (2.23g, 12.3mmol), and K_2CO_3 (6.82g, 49.3mmol) in 40 mL of butanone was heated at 80°C for overnight. The reaction solution was filtered to remove inorganic material and then evaporated. The crude product was purified using flash chromatography on silica gel (70% ethyl acetate in hexane, r.f=0.4) to give a yellow solid (1.65g, 55% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.47 (m, 4H, $HOCH_2CH_2CH_2CH_2CH_2CH_2O$), 1.60 (quintet, 2H, J=6.6Hz, CH_2CH2O), 1.81 (quintet, 2H, J=6.6Hz, $HOCH_2CH_2$), 3.66 (t, 2H, J=6.6Hz, $HOCH_2$), 3.97 (t, 2H, J=6.6Hz, CH_2O), 6.40 (d, 1H, J=9.6Hz, C*CH*=CHCOO), 6.88 (d,1H, J=3.0Hz, CCH=*CH*COO), 7.08 (d.d, 1H, J=3.0Hz, J=9.0Hz, Ar), 7.24 (d, 1H, J=9.0Hz, Ar), 7.62 (d, 1H, J=9.6Hz, Ar).

2.4 Preparation of 6-((6-bromohexyl)oxy)coumarin

To a solution of 6-((6-hydroxyhexyl)oxy) coumarin (100 mg, 0.38mmol) and 1,2-bis(diphenylphosphino)ethane (DIPHOS, 182 mg 0.46 mmol) in 3 mL of dried CH_2Cl_2 was slowly added dropwise a solution of carbon tetrabromide (152 mg. 0.46 mmol) in 1 mL of dried CH_2Cl_2 at 0°C under argon. The reaction mixture was stirred for 1 hour at 0°C under argon, and then diluted with CH_2Cl_2. The resulting solution of the reaction mixture was filtered through celite. The filtrate was evaporated at reduced pressure and the residue was purified by flash chromatography on silica gel (33% ethyl acetate in hexane, r.f=0.5) to give a white solid (111mg, 90% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.52 (m, 4H, $BrCH_2CH_2CH_2CH_2CH_2CH_2O$), 1.60 (quintet, 2H, J=6.6Hz, CH_2CH2O), 1.88 (m, 4H, $BrCH_2CH_2CH_2CH_2CH_2CH_2O$), 3.44 (t, 2H, J=6.6Hz, $BrCH_2$), 3.97 (t, 2H, J=6.6Hz, CH_2O), 6.43 (d, 1H, J=9.6Hz, C*CH*=CHCOO), 6.92 (d,1H, J=3.0Hz, CCH=*CH*COO), 7.11 (d.d, 1H, J=3.0Hz, J=9.0Hz, Ar), 7.26 (d, 1H, J=9.0Hz, Ar), 7.65 (d, 1H, J=9.6Hz, Ar).

2.5 Preparation of 6-((6-azidohexyl)oxy)coumarin

A stock solution of 0.25M NaN_3 in dry DMSO was prepared by stirring the solution for 24 hours at 25°C. To a 10 ml round-bottom flask equipped with a magnetic stir bar, was added a 0.25M solution of NaN_3 (24.4mg, 0.38mmol) in DMSO (1.5mL) at 25°C. To this solution was added 6-((6-bromohexyl)oxy)coumarin (100 mg, 0.31mmol), and the mixture was stirred 3 hours at room temperature. The reaction was quenched with D.I. H_2O (3.0 mL) [slightly exothermic] and stirred until it cooled to room temperature. The reaction was extracted with Et_2O (3x15mL); the Et_2O extracts were washed with H_2O (2x15mL) and once with brine (15mL). The organic layer was dried ($MgSO_4$) filtered, and the solvent removed under vacuo (20 Torr) to afford the pure alkyl azide (Azidocoumarin) in almost quantitative yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.49 (m, 4H, $N_3CH_2CH_2CH_2CH_2CH_2CH_2O$), 1.59 (quintet, 2H, J=6.6Hz, CH_2CH2O), 1.77 (m, 4H, $N_3CH_2CH_2CH_2CH_2CH_2CH_2O$), 3.26 (t, 2H, J=6.6Hz, N_3CH_2), 3.96 (t, 2H, J=6.6Hz, CH_2O), 6.43 (d, 1H, J=9.6Hz, C*CH*=CHCOO), 6.90 (d,1H, J=3.0Hz, CCH=*CH*COO), 7.12 (d.d, 1H, J=3.0Hz, J=9.0Hz, Ar), 7.26 (d, 1H, J=9.0Hz, Ar), 7.65 (d, 1H, J=9.6Hz, Ar).

2.6 Preparation of 9-(azidomethyl)antheracene

50 mg of 9-(chloromethyl)antheracene was dissolved in 10 ml of dry DMF followed by the careful addition of NaN_3 (100 mg). The reaction mixture was stirred at room

temperature for 12 hours in a dark place. Final product (Azidoanthracene) was washed and centrifuged 5 times by cold solution of D.I. water and ethanol (4:1 v/v) and finally 5 times by D.I water to ensure the removal of all the residual DMF and NaN_3.

2.7 Synthesis of Alkyne Bearing CNC Derivative

TEMPO-oxidized CNCs (50mg) were mixed in 6 mL of MES [2-(*N*-morpholino)-ethanesulfonic acid buffer (50 mM, pH = 4). Resulting suspension was further treated with ultrasound treatment (5 min, 20% power with 30% pulser on; Omni-Ruptor 250 Ultrasonic Homogenizer, Omni International Inc.) to break down the cellulose aggregates. In typical synthesis, 120 mg of EDC·HCl [*N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride)], 72 mg of NHS (*N*-hydroxysuccinimide), and 60 μL of propargylamine, respectively, were added to the CNC suspension [38]. The reaction was performed at room temperature under stirring for 24 h. The resulting mixture was dialyzed (cutoff = 12 kDa) against a saturated NaCl solution for 1 day and then against distilled water for 3 days. Finally, the CNC-PR derivative (see Scheme 1, step 2) was recovered by freeze-drying.

2.8 Synthesis of Coumarine Click Product

A 25 mg amount of CNC-PR and a 25 mg of azidocoumarin were mixed in 3.0 mL of mixture of EtOAc : MeOH : H_20 (1:1:1). Next, 25 μL of $CuSO_4$ x $5H_2O$ aqueous solution (7.5% w/v) and 30 μL of Ascorbic acid (1M sol.) were added and the mixture was vigorously stirred in the dark overnight at the room temperature leading to a formation of coumarin modified CNCs (see Scheme 1, step 3). Finally, the CNC-Coumarin was extensively washed with cold water and dichloromethane to remove the inorganic catalyst and the excess azidocoumarin.

2.9 Synthesis of Anthracene Click Product

A 50 mg amount of CNC-PR and 25 mg of 9-(azidomethyl)antheracene were mixed in 10.0 mL of ethanol. Next, a mixture of 5 mg CuSO4 x $5H_2O$ and 15 mg of ascorbic acid in 0.5 ml water were added and the mixture was vigorously stirred for 5 min. The stirring of the mixture was continued in the dark for 8 hours at the room temperature leading to a formation of anthracene modified CNCs (see Scheme 1, step 4). Finally, the product was washed 3 times with DMF, 5 times with water and then freeze dried.

2.10 Benzoylation of Cellulosic samples

The method described below was developed by the group of Argyropoulos following the principles established in the work described elsewhere [39]. Ionic liquid, 1-allyl-3-methylimidazolium chloride ([Amim]Cl, 950 mg) were added to CNC (50 mg) in a 15 ml sample flasks, vortexed until all solid particles had dispersed and heated at 80°C with magnetic stirring until the solutions were transparent (2 hrs). Pyridine (330 μl, 3.7 mmol) was added and the solution vortexed until homogeneous and allowed to cool down to room temperature. Benzoyl Chloride (380 μl, 3.3 mmol) was added in one portion and the resulting mixture was vortexed until the formation of homogeneous white paste. The sample was then heated at 40 °C for 3 hrs with magnetic stirring and then allowed to cool down to the room temperature. Next, the mixture of deionized water (2.5 ml) and EtOH (7.5 ml) was added and the mixture vigorously shaken and vortexed for 5 mins. The solid was filtered off through a sintered funnel (grade M), washed further with EtOH and purified with MeOH (stirred overnight without heating). Finally, the resulting solid was filtered off to give a white powder.

2.11 Infrared Spectroscopy

FTIR spectra were measured on a Thermo Nicolet NEXUS 670 FT-IR infrared spectrophotometer. Spectra in the range of 4000 – 650 cm^{-1} were obtained with a resolution of 4 cm^{-1} by cumulating 64 scans. In prior to analysis a small amount of TEMPO-oxidized material was acidified with dilute HCl in order to convert carboxylates to the free carboxylic acid form. Subsequently, the degree of oxidation (DO) measurements were carried out by comparing the intensities of absorption band near 1730 cm^{-1} (carbonyl stretching frequency) to that of 1050 cm^{-1} (cellulose backbone) [40].

2.12 Acid-base Titration

The carboxylic acid content of the oxidized CNCs was determined by acid-base titration following the procedure developed for the conductometric titrations of such materials [38]. In this procedure tempo-oxidized CNC samples (50mg) were suspended into 0.01 M hydrochloric acid (HCl) solutions (15 ml) with stirring. The resulting suspensions were then titrated with 0.01 M sodium hydroxide (NaOH) solution. The degree of oxidation (DO) value was calculated as already published and very reproducible results were obtained [41].

2.13 Fluorescence Microscopy

Microscope: Olympus BX 51, Objective 40x 0.75 NA, Camera: Q-Imaging Micropublisher 3.3 Excitation: 350/50nm Emission: 460/50 nm Dichroic Beamsplitter: 400 DCLP. The 3D images were taken using Zeiss LSM 710 confocal microscope with the 20x 0.8 NA objective.

2.14 UV Light Absorption

Experiments were carried out in DMSO by monitoring the wavelength range from 200 to 500 nm.

2.15 Elemental Analysis

The percent carbon (C), hydrogen (H) and nitrogen (N) contents (%) of unreacted TEMPO-oxidized cellulose nanocrystals, its alkyne and anhtracene derivatives were determined by Perkin Elmer element analyzer (Norwalk, CT, USA). The remaining sample was assumed to be oxygen (O).

2.16 ^{1}HNMR Spectroscopy

NMR measurements were acquired using on a Bruker 300 MHz spectrometer equipped with a Quad probe dedicated to ^{31}P, ^{13}C, ^{19}F, and ^{1}H acquisition.

2.17 GPC Analysis

Gel permeation chromatographic (GPC) measurements were carried out with a Waters GPC 510 pump equipped with UV and RI detectors using THF as the eluent at a flow rate of 0.7 mL/min at 40°C. Two Ultrastyragel linear columns (Styragel HR 1 and Styragel HR 5E), connected in series, were used for the measurements. Standard mono-disperse polystyrenes with molecular weight ranges from 0.82 to 1860 kg/mol were used for calibration. The number- and weight-average molecular weights were calculated using the Millenium software of Waters.

2.18 Photochemical irradiation

Aqueous dispersion of cellulosic samples were disposed to a glass plate and let stand overnight. The resulting films were irradiated with a 75W xenon lamp at variable times (6hr and 48hr) for coumarine-CNC derivatives and 12 hrs for anthracene-CNC derivatives at room temperature.

3. Results and Discussion

TEMPO-oxidized cellulose nanocrystals (CNCs) have been reacted with propargylamine in the presence of the coupling agent N-hydroxysuccinimide to form a precursor suitable for a Click chemistry reaction (Scheme 1). The Click reaction was performed with the modified azide-bearing coumarin and anthracene chromophores which were synthesized starting from 6-hydroxycoumarin and 9-(chloromethyl)antheracene, respectively.

3.1 FTIR Analysis of the TEMPO-oxidized CNCs

In the first step, cellulose nanocrystals were oxidized by using TEMPO-mediated hypohalite oxidation. TEMPO-oxidation selectively converts the primary hydroxyl groups of cellulose to carboxylic acids. Therefore, TEMPO-oxidation provides known pattern of reactive sites for further derivatization reactions. FTIR spectra of TEMPO-oxidized cellulose nanocrystals contains a band around 1730 cm^{-1} which corresponds to the C=O stretching frequency of carboxyl groups in their acidic forms. The degree of oxidation (DO) values was roughly estimated by comparing the intensity of the new band at 1730 cm^{-1} to that near 1050 cm^{-1} which derives from the cellulose backbone [40]. FTIR analysis led to a DO value of 0.21 for the oxidized CNCs. This value is in good agreement with the DO value of 0.19 achieved by the acid-base titration of TEMPO-oxidized CNCs [41]. It should be noted here that an average DO value of 0.2 is used for the calculations throughout the manuscript.

3.2 Elemental Analysis of the Fluorescence Click Derivatives

The nitrogen content of CNC-PR confirmed the successful grafting reaction (Table 1). In order to outsource the possibility of the nitrogen deriving from the used crosslinking agents (1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride and *N*-hydroxysulfosuccinimide) the TEMPO-oxidized CNCs were subjected to identical reaction conditions with the absence of the derivatization compound (propargylamine). It is worth to mention here that the content of oxygen is elevated in TEMPO-oxidized CNCs when compared to the CNCs, indicating the successful oxidation reaction. Moreover, the nitrogen content of CNC-PR was found to be higher than that of the starting material (TEMPO-ox. CNCs).

Sample	% C	% H	% N	% O[a]
CNCs	43.6	6.1	0.04	50.3
TEMPO-ox. CNCs	41.2	5.7	0.08	52.4
CNC-PR	43.2	5.2	0.79	50.7
CNC-Anthracene	49.5	7.1	1.6	41.8

[a] O = 100 % - C (%) – H (%) – N (%)

Table 1. Carbon, hydrogen, oxygen and nitrogen contents of cellulosic samples

The grafting density of synthesized precursor (CNC-PR) was calculated based on the DO value (0.2) of the TEMPO-oxidized CNCs. The DO value 0.2 means that

20% of the hydroxymethyl groups on cellulose have been oxidized to corresponding carboxylic acid groups and are thus susceptible for the subsequent grafting reactions. Therefore, the maximum grafting density corresponds to the situation where every fifth of the anhydroglucose units in cellulose contain a grafted propargylamine moiety. As a result, the completely grafted TEMPO-oxidized CNCs should contain 1.6% of nitrogen [14 / (5 x 162 + 40)]. However, the amount of nitrogen found in the precursor (CNC-PR) was 0.79% which corresponds to the grafting density of ~50% i.e. half of the available carboxyl groups were grafted with propargylamine i.e. every tenth of the anhydroglucose units in cellulose contained a grafted propargylamine moiety. This corresponds to the overall degree of substitution (DS) value of 0.1 on cellulose polymer. Incomplete grafting is not totally surprising since the reactions were carried out under heterogeneous conditions.

The grafting density of anthracene modified CNCs (CNC-Anthracene) was calculated based on the grafting density of its precursor (CNC-PR). Therefore, the maximum grafting density corresponds to the situation where every tenth of the anhydroglucose units in cellulose contain a grafted anthracene moiety. As a result, the completely grafted CNC-PR should contain 3.01% of nitrogen [4x14 / (10 x 162 + 238)]. However, the amount of the nitrogen found in CNC-Anthracene was 1.59% which corresponds to the grafting density of ~50% i.e. half of the available propargyl groups were grafted with 9-(azidomethyl)antheracene i.e. every twentieth of the anhydroglucose units in cellulose contained a grafted anthracene moiety. This corresponds to the overall degree of substitution (DS) value of 0.05 on cellulose polymer. In addition the DS value was also calculated by using the carbon content (%C) of CNC-Anthracene (Table 1) following the previously published method [42]. This method led to a DS value of 0.048 which is in good agreement with the results obtained by using the nitrogen (%N) content.

The decision to perform the click reaction between propargyl-modified CNCs (CNC-PR) and azidocoumarin is based on the fact that already fluorescent azidocoumarin will provide additional fluorescence upon a formation of 1,2,3-triazole product as a result of the CuI-catalyzed 1,3-dipolar cycloaddition reactions with terminal alkynes [43, 44]. Subsequently, the resulting product (CNC-Coumarin) should be highly fluorescent, and a successful derivatization will be clearly demonstrated. The reaction between modified CNCs (CNC-PR) and azidocoumarin proceeds smoothly in aqueous media and gives the corresponding highly fluorescent triazole-modified CNCs.

3.3 Fluorescence Microscopy of the Fluorescence Click Derivative

The modified CNCs (CNC-Coumarin) are intensely blue fluorescent, as shown by a fluorescence photomicrograph (Figure 1c).

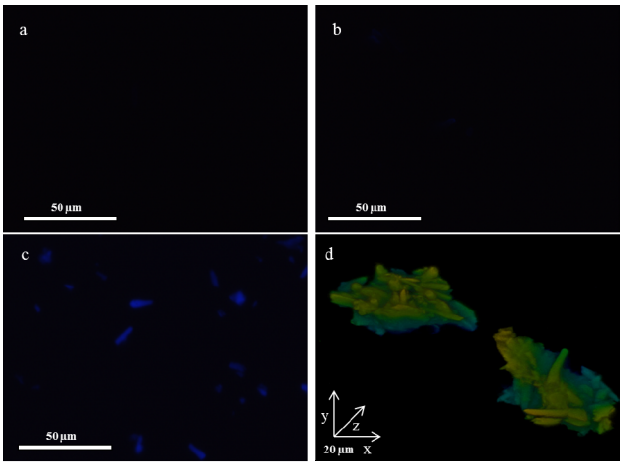


Figure 1. a) Fluorescence photomicrograph of TEMPO-oxidized cellulose nanocrystals. b) Fluorescence photomicrograph of the cellulose material after the reaction between TEMPO-oxidized cellulose nanocrystals and azidocoumarin (negative control). c) Fluorescence photomicrograph of CNC-Coumarin dispersed in water. d) 3D reconstructions of 45 images taken at different focus levels (CNC-Coumarin).

It is worth to mention here that both succinimide assisted amidation and the CuI-catalyzed 1,3-dipolar cycloaddition are relatively mild reaction conditions for the cellulose nanocrystal modifications. Moreover, the click reaction confirms that CNCs has been grafted with propargylamine, since the copper catalyzed cycloaddition between unmodified TEMPO-oxidized CNCs and azidocoumarin does not provide a fluorescent CNC product (Figure 1b). In fact, it appears as non-fluorescent as TEMPO-oxidized CNCs (Figure 1a).

The size distribution of the fluorescent cellulose particles (CNC-Coumarin) in water dispersion is rather broad varying from 2 to 15 µm in length and 2 to 5 µm in width (Figure 1c). It is important to note that the fluorescent particles are significantly larger than the starting Tempo-oxidized CNCs that possessed nanometer dimensions. This could be due to the aggregation of the derivatized cellulose particles and/or possible [2+2] cycloaddition between the grafted coumarin side chains which can connect individual nanocrystals to larger particles. The 3D confocal microscopic image of dry state CNC-Coumarin revealed even larger particles having approximate dimensions of 60 x 30 x 20 µm (Figure 1d). Moreover, the 3D constructions clearly displayed the formation of bulkier cellulose components as a result of the aggregation of the smaller particles.

www.intechweb.org
www.intechopen.com

3.4 UV Absorptions of CNC-Anthracene and Its Photodimerized Derivative

Anthracene has typical UV light absorption bands at 392 nm, 372 nm, 355 nm and 338 nm, respectively. UV light absorption spectra of CNCs, anthracene and CNC-Anthracene reveal that anthracene modified CNCs have the same absorption bands than those of the control anthracene whereas in the case of the unmodified CNCs no UV absorption was observed in the investigated region (Figure 2). This clearly points toward a successful grafting reaction between anthracene and CNCs.

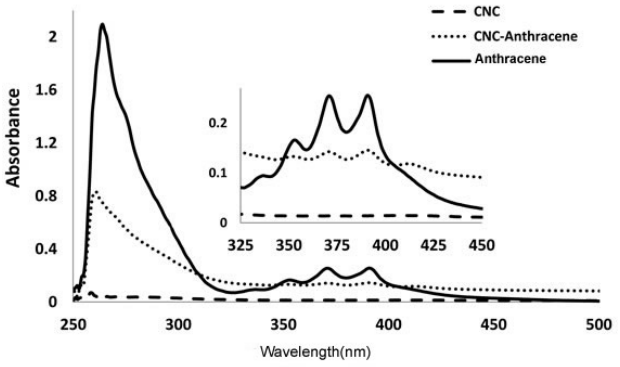


Figure 2. UV light absorption spectra of anthracene (solid line), CNC-Anthracene (dotted line) and CNCs (dashed line).

The effects of the UV irradiation on CNC-Anthracene were investigated by comparing the photodimerization of anthracene to that of CNC-Anthracene. It can clearly be seen from Figure 3 that the typical absorption bands of anthracene are disappearing as a result of the photodimerization triggered by the UV irradiation.

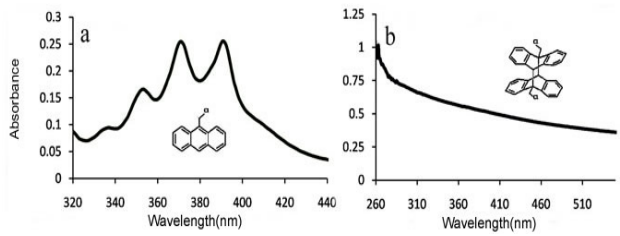


Figure 3. UV light absorption spectra of anthracene (a) and photodimerized anthracene (b).

Similarly, the photoirradiation of CNC-Anthracene results in the disappearance of the UV absorption bands indicative of the photodimerization of CNC-Anthracene (Figure 4). It is worth to mention here that such stimuli responsive performance is highly desired as far as the controlled assembly of the supramolecular structures is concerned.

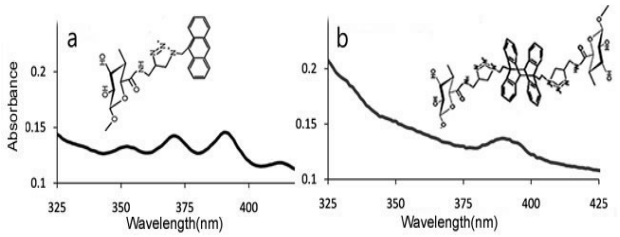


Figure 4. UV light absorption spectra of CNC–Anthracene (a) and photoirradiated CNC–Anthracene (b).

3.5 GPC Analysis of Fluorescence Click Derivative

The molecular weight distributions of UV irradiated TEMPO-oxidized CNCs (TEMPO-ox. CNC 6hr) and CNC-Coumarin (CNC-Coumarin 6hr, CNC-Coumarin 48hr) and CNC-Anthracene (12 hr) were investigated by size exclusion chromatography. Due to the insolubility of cellulosic samples in tetrahydrofuran (THF), all hydroxyl groups on them were benzoylated prior to the analysis. In order to achieve a high degree of benzoylation, the reactions were carried out in ionic liquid, 1-allyl-3-methylimidazolium chloride ([Amim]Cl), that is known to be able to dissolve cellulosic materials. As expected, the molecular weight of the UV irradiated (6hr) CNC-coumarin is somewhat higher than that of the TEMPO-oxidized CNCs indicating that the UV induced [2+2] cycloaddition reaction of the installed coumarin side chains has linked individual CNCs together (Figure 5, Table 2).

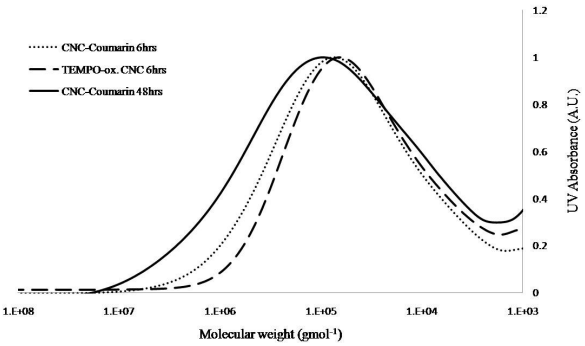


Figure 5. Gel permeation chromatograms of irradiated cellulosic samples (CNC-Coumarin 6hrs, dotted line), (TEMPO-ox. CNC 6hrs, dashed line) and (CNC-Coumarin 48hrs, solid line).

Interestingly, the elevated irradiation time (48hr) was seen to further increase the molecular weight of CNC-Coumarin thus pointing out the effect of irradiation time for the degree of [2+2] cycloaddition reaction. As can be seen from the data of Table 2, the longest irradiation time (48hr) increased the weight average molecular weight of CNC-Coumarin approximately 4 fold when compared to its precursor (TEMPO-ox. CNC). Moreover, the significantly elevated ratio of Mw/Mn of CNC-Coumarin is characteristic for polydisperse materials.

Sample	Mw (1 x10^3 $gmol^{-1}$)	Polydispersity (PD)
TEMPO-ox. CNC (6hrs Irradiation)	85	4.3
CNC-Coumarin (6hrs Irradiation)	141	7.6
CNC-Coumarin (48hrs Irradiation)	373	16.3
CNC-Anthracene (12 hrs Irradiation)	139	6.7

Table 2. Molecular weight distributions of irradiated cellulosic samples

The number average molecular weight (M_w) of CNC-Anthracene was found to increase from 85x10^3 g/mol to 139x10^3 g/mol during 12 hours of UV irradiation (Figure 6, Table 2). This clearly indicates that the photoinduced [4+4] cycloaddition reaction has taken place and subsequently linked individual CNC-Anthracene molecules together. It is worth to mention here that the increase in the molecular weight was found to be almost identical with that of the CNC-Coumarin after 6 hours of UV-irradiation. Photodimerization can occur in two different pathways: (1) intramonolayer photodimerization between coumarin/athracene groups attached in the same cellulose nanocrystal and (2) interfacial photodimerization between coumarin or anthracene groups attached to different cellulose nanocrystals [45]. It should be noted here that the intramonolayer pathway is not expected to increase the molecular weight of the irradiated material whereas the interfacial pathway brings the individual molecules together and thus the molecular weight is increased. Therefore, the observed increases in the molecular weights of UV irradiated CNC-Coumarin and CNC-Anthracene point towards the interfacial photodimerization mechanism. However, it is important to note here that the DS values of CNC-Coumarin and CNC-Anthracene were rather low which in turn means that the photoactive coumarin/anthracene sidechains are not proximal to each other. Therefore, one possible explanation for the predominant interfacial photodimerization could be the small amount of photoactive groups along the surface of cellulose nanocrystals thus diminishing the intramonolayer reaction. Additional control in the observed photodimerization reaction is currently explored in our laboratory by preparing photoactive cellulose nanocrystals possessing higher DS values as well as by varying the UV irradiation exposure times. In addition, the reversibility of the photocoupling is currently under investigation.

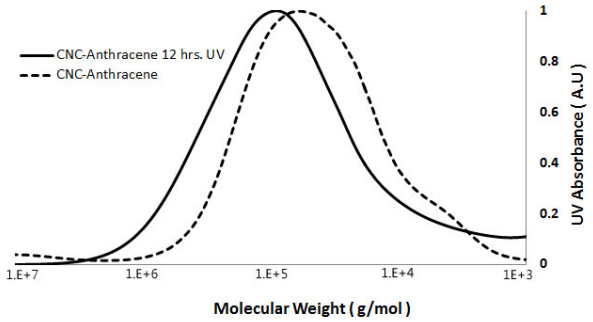


Figure 6. Gel permeation chromatograms of CNC-Anthracene (dashed line) and irradiated (12hrs) CNC-Anthracene (solid line)

4. Conclusions

Click chemistry has been utilized for the preparation of new fluorescent and photoresponsive cellulose nanomaterials. The primary hydroxyl groups in cellulose nanocrystals were first selectively oxidized to carboxylic acids. These carboxylic acid functionalities were further used as reactive sites for the amidation reaction that provided the precursor necessary for the final linking of the fluorescent coumarin and anthracene probes into the cellulose surface. Furthermore, UV irradiation of the produced materials was found to trigger the photodimerization of the installed side chains bringing together the individual nanocrystals.

5. Acknowledgments

The authors would like to thank the *College of Natural Resources at NCSU* for the award of the Hofmann Fellowship to one of us (IF) that made graduate studies possible.

6. References

[1] C. Y. Yin, J. B. Li, Q. Xu, Q. Peng, Y. B. Liu, X. Y. Shen, "Chemical modification of cotton cellulose in supercritical carbon dioxide: Synthesis and characterization of cellulose carbamate," *Carbohydr. Polym.*, vol. 67, pp. 147-154, 2007.

[2] S. P. Dong, M. Roman, "Fluorescently labeled cellulose nanocrystals for bioimaging applications," *J. Am. Chem. Soc.*, vol. 129, pp. 13810-13811, 2007.

[3] E. Siegel, K. Schundehutte, D. Hildebrand, *Reactive dyes: Reactive groups* (The Chemistry of Synthetic Dyes). Venkataraman K, Ed., vol. 6, Chapter 1. New York, Academic Press, 1972, pp. 2-108.

[4] H. R. Hensel, G. Luetzel, "Reactions of metal acetylacetonates," *Angew Chem Int Ed.*, vol. 4, pp. 312-318, 1965.

[5] R. D. Gilbert, P. A. Patton, "Liquid-crystal formation in cellulose and cellulose derivatives," *Prog. Polym. Sci.*, vol. 9, pp. 115-131, 1983.

[6] P. Haurand, P. Zugenmaier, "Structure and phase behavior of a lyotropic mesophase system: cellulose tricarbanilate-methyl acetoacetate," *Polymer*, vol. 32, pp. 3026-3037, 1991.

[7] N. Yoshiyuki, C. Ryotaro, "Structural characteristics and novel functionalization of liquid-crystalline polysaccharides and cholesterol derivatives," *Ekisho*, vol. 7, pp. 218-227, 2003.

[8] C. T. Yim, D. F. R. Gilson, T. Kondo, D. G. Gray, "Order parameters and side-chain conformation in ethyl cellulose/chloroform liquid crystal phases," *Macromolecules*, vol. 25, pp. 3377-3380, 1992.

[9] W. Burchard, "Solubility and Solution Structure of Cellulose Derivatives," *Cellulose*, vol. 10, pp. 213-225, 2003.

[10] A. Isogai, A. Ishizu, J. Nakano, "Preparation of tri-O-substituted cellulose ethers by the use of a nonaqueous cellulose solvent," *J. Appl. Polym. Sci.*, vol. 29, pp. 3873-3882, 1984.

[11] K. Arai, H. Udagawa, "Application of photoresponsive groups-containing cellulose as an adsorbent for thin layer chromatography," *Makromol. Chem. Rapid Commun.*, vol. 9, pp. 797-800, 1988.

[12] X. Tang, L. Gao, X. Fan, Q. Zhou, "Controlled grafting of ethyl cellulose with azobenzene-containing polymethacrylates via atom transfer radical polymerization," *J. Polym. Sci., Part A: Polym. Chem.*, vol. 45, pp. 1653-1660, 2007.

[13] K. Yang, S. Yang, J. Kumar, "Formation mechanism of surface relief structures on amorphous azo-polymer films," *Phys. Rev. B: Condens. Matter Mater. Phys.*, vol. 73, pp. 1-14, 2006.

[14] K. Arai, "Development of cellulosic optically functional materials," *Kami Pa Gikyoshi*, vol. 46, pp. 969-980, 1992.

[15] E. Yashima, J. Noguchi, Y. Okamoto, "Photocontrolled Chiral Recognition by [4-(Phenylazo)phenyl]carbamoylated Cellulose and Amylose Membranes," *Macromolecules*, vol. 28, pp. 8368-8374, 1995.

[16] C. Wu, Q. Gu, Y. Huang, S. Chen, "The synthesis and thermotropic behaviour of an ethyl cellulose derivative containing azobenzene-based mesogenic moieties," *Liq. Cryst.*, vol. 30, pp. 733-737, 2003.

[17] K. Arai, H. Udagawa, "Photoregulation of liquid crystalline phase of cellulose containing azobenzene moiety," *Sen'i Gakkaishi*, vol. 46, pp. 150-154, 1990.

[18] J. Hafrén, W. Zou, A. Córdova, "Heterogeneous 'Organoclick' derivatization of polysaccharides," *Macromol. Rapid Comm.*, vol. 27 pp. 1362-1366, 2006.

[19] K. K. Kumar, R. M. Kumar, V. Subramanian, T. M. Das, "Expedient synthesis of coumarin-coupled triazoles via 'click chemistry' leading to the formation of coumarin–triazole–sugar hybrids," *Carbohydr. Res.*, vol. 345, pp. 2297–2304, 2010

[20] Mallard, D. Landy, N. Bouchemal, S. Fourmentin, "Synthesis and inclusion ability of anthracene appended b-cyclodextrins:unexpected effect of triazole linker," *Carbohydr. Res.*, vol. 346, pp. 35–42, 2011.

[21] C. Sluszny, V. Bulatov, V. V. Gridin, I. Schechter, "Photochemical Study of Anthracene Crystallites by Fourier Transform Spectroscopic Imaging," *Photochem. Photobiol.*, vol. 74, pp. 780-786, 2001.

[22] S. Nakatsuji, T. Ojima, H. Akutsu, J. Yamada, "Anthracene Derivatives and the Corresponding Dimers with TEMPO Radicals," *J. Org. Chem.*, vol. 67, pp. 916-921, 2002.

[23] G. W. Breton, X. Vang, "Photodimerization of Anthracene. A [4p+4p] Photochemical Cycloadddition," *J.Chem.Ed.*, vol. *75*, pp. 81–82, 1998.

[24] H. D. Becker, "Unimolecular photochemistry of anthracenes," *Chem. Rev.*, vol. 93, pp. 145-172, 1993.

[25] H. Bouas-Laurent, A. Castellan, J.-P. Desvergne, R. Lapouyade, "Photodimerization of anthracenes in fluid solutions: (part 2) mechanistic aspects of the photocycloaddition and of the photochemical and thermal cleavage," *Chem. Soc. Rev.*, vol. 30, pp. 248-263, 2001.

[26] H. Bouas-Laurent, A. Castellan, J.-P. Desvergne, R Lapouyade, "Photodimerization of anthracenes in fluid solution:structural aspects," *Chem. Soc. Rev.*, vol. 29, pp. 43-55, 2000.

[27] N. Iza, M. Gil, J. L. Montero, J. A. Morcillo, " study of anthracene-metallic cation interactions by UV-Vis spectroscopy," *J. Mol. Str.*, vol. 175, pp. 323-328, 1988.

[28] D. S. Tyson†, A. D. Carbaugh, F. Ilhan, J. Santos-Pérez, M. A. Meador, "Novel Anthracene Diimide Fluorescent Sensor," *Chem. Mater.*, vol. 20 pp. 6595-6596, 2008.

[29] Y.C. Hsieh, J.L. Chir, H.H. Wu, P.S. Chang and A.T. Wu, "A sugar-aza-crown ether-based fluorescent sensor for Hg^{2+} and Cu^{2+}," *Carbohydr. Res.*, vol. 344, pp. 2236–2239, 2009.

[30] Y. Sun, C. Zhong, R. Gong, H. Mu, E. Fu, "A ratiometric fluorescent chemodosimeter with selective recognition for sulfite in aqueous solution, " *J. Org. Chem.*, vol. 74, pp. 7943–7946, 2009.

[31] C. Kim, K.-I. Lim, C.-G. Song, "Diels–Alder reaction of anthracene on carbosilane dendrimer, " *J. Organomet. Chem.*, 2005, vol. 690, pp. 3278-3285, 2005.

[32] C. Bratschkov, "FTIR spectroscopy study of the UV irradiation changes in an anthracene containing copolymer," *European Polymer Journal.*, vol. 37, pp. 1145-1149, 2001.

[33] A. Bezrukova, M. Lubomska, M. Rogalski, " Nanoparticle Mixtures of Anthracene and β-Cyclodextrin Testing by Optical Spectroscopy, " *Rev.Adv.Mater.Sci.*, vol. 20, pp. 70-76, 2009.

[34] R.K. Iha, K.L. Wooley, A.M. Nystrom, D.J. Burke, M.J. Kade, C.J. Hawker, "Applications of orthogonal "Click" Chemistries in the synthesis of functional soft materials," *Chem. Rev.*, vol. **109**, pp. 5620–5686, 2009.

[35] J. Araki, M. Wada, S. Kuga, T. Okano, "Influence of surface charge on viscosity behavior of cellulose microcrystal suspension," *J. Wood Sci.,* vol. 45, pp. 258-261, 1999.

[36] J. Araki, M. Wada, S. Kuga, "Steric stabilization of a cellulose microcrystal suspension by polyethylene glycol (PEG) grafting," *Langmuir*, vol. 17, pp. 21-27, 2001.

[37] J. Araki, M. Wada, S. Kuga, T. Okano, "Flow properties of microcrystalline cellulose suspension prepared by acid treatment of native cellulose," Colloids Surf., A, vol. 142, pp. 75–82, 1998.

[38] Filpponen, D. S. Argyropoulos, "Regular Linking of Cellulose Nanocrystals via Click-Chemistry: Synthesis and Formation of Nano-Platelet gels," *Biomacromolecules*, vol. 11, pp. 1060-1066, 2010.

[39] H. Xie, A. King, I. Kilpelainen, M. Granstrom, D. S. Argyropoulos, "Thorough chemical modification of wood-based lignocellulosic materials in ionic liquids," *Biomacromolecules*, vol. 8, pp. 3740-3748, 2007.

[40] Y. Habibi, H. Chanzy, M. R. Vignon, "TEMPO-mediated surface oxidation of cellulose whiskers," *Cellulose,* vol. 13, pp. 679-687, 2006.

[41] D. Da Silva Perez, S. Montanari, M. R. Vignon, "TEMPO-Mediated Oxidation of Cellulose III," *Biomacromolecules*, vol. 4, pp. 1417-1425, 2003.

[42] M. Krouit, R. Granet, P. Krausz, "Photobactericidal plastic films based on cellulose esterified by chloroacetate and a cationic porphyrin," *Bioorg Med Chem*., 2008 vol. 16, pp. 10091-10097, 2008.

[43] K. Sivakuman, F. Xie, B. M. Cash, S. Long, H. N. Barnhill, Q. Wang, "A Fluorogenic 1,3-Dipolar Cycloaddition Reaction of 3-Azidocoumarins and Acetylenes," *Org. Lett*., vol. 6, pp. 4603-4606, 2004.

[44] K. E. Beatty, F. Xie, Q. Wang, D. A. Tirrell, "Selective Dye-Labeling of Newly Synthesized Proteins in Bacterial Cells," *J. Am. Chem. Soc.,* vol. 127, pp. 14150-14151, 2005.

[45] A. R. Smith, D. F. Watson, "Photochemically Triggered Assembly of Composite Nanomaterials through the Photodimerization of Adsorbed Anthracene Derivatives," *Chem. Mater.,* vol. 22, pp. 294-304., 2010.