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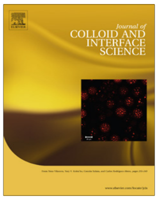

Synthesis and characterization of pH-responsive and fluorescent poly (amidoamine) dendrimer-grafted cellulose nanocrystals

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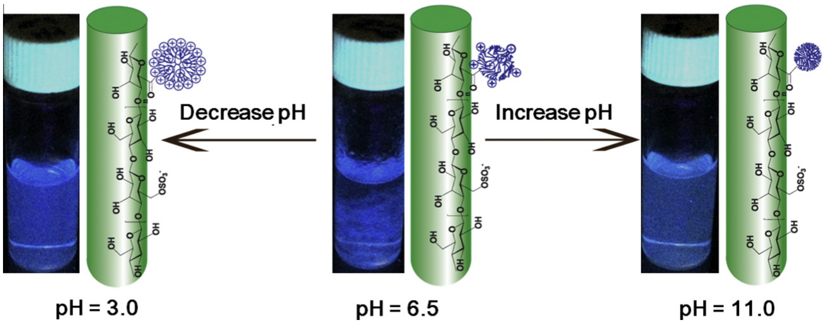
GRAPHICAL ABSTRACT



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ABSTRACT

Poly (amidoamine) (PAMAM) dendrimers have found promising applications in biomedicine and in the encapsulation of inorganic nanoparticles. G6 PAMAM dendrimer-grafted cellulose nanocrystals (CNC-PAMAM) were prepared via a simple carbodiimide-mediated amidation process and they displayed pH-responsive and fluorescent characteristics as confirmed by zeta potential, transmittance, isothermal titration calorimetry (ITC), and fluorescence spectroscopy. Stable aqueous dispersions of CNC-PAMAM were obtained at pH $\leqslant 4$ and pH $\geqslant 10$, driven by electrostatic repulsion from positive charge and negative charge respectively. However, large aggregates were formed at pH values from 5 to 9 due to electrostatic attraction. In addition, strong blue fluorescent emission was observed, and the fluorescent behaviour of CNC-PAMAM was influenced by the formation of aggregates. The pH-responsive and fluorescent properties of CNC-PAMAM may be suitable for their applications in pH-responsive nanodevices, fluorescent-based pH sensors, optical markers, and nanoreactors for the encapsulation of inorganic nanoparticles.

1. Introduction

Poly (amidoamine) (PAMAM) dendrimers have attracted increasing attention due to their well-defined unique dendritic architecture, controllable size, mimicry of globular proteins, encapsulation capability, favourable surface modification, and commercial availability [1,2]. PAMAM (full generation) contains abundant amounts of primary amines on the surface as well as tertiary amines in the interior. The protonation/deprotonation process of these two amino groups occurs at different pH ranges permitting one to tune their pH-responsive property [3]. Specifically, PAMAM structures are stretched yielding a highly ordered open structure at pH $\leqslant 4$, while they are globular and compressed at pH $\geqslant 10$ [4,5]. This pH-responsive property enables its use as macromolecular vector [6,7] and nanoreactor for

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encapsulating inorganic nanoparticle [8]. In addition, intrinsic fluorescence from carboxylate- [9], amine- [10], and hydroxy-terminated [11] PAMAM dendrimers have been reported, which leads to their potential use in fluorescent-based detection and diagnostics. For example, intrinsic fluorescent PAMAM dendrimers were recently applied as gene carriers and nanoprobes for monitoring the delivery of nucleic acids [12].

PAMAM dendrimers have been grafted onto various templates and substrates, such as on multi-walled carbon nanotubes [13], iron oxide, silica nanoparticles [14,15], silica support [16], and indium tin oxide electrodes [17] for use in both biomedical and catalytic applications. Here we report on the functionalization of an excellent biotemplate–cellulose nanocrystals (CNCs) with PAMAM dendrimers. CNCs continue to attract increasing attentions due to their excellent mechanical strength, rigidity, high specific area, biocompatibility, biodegradability, sustainability and commercial availability [18]. In addition, the abundance of hydroxyl groups on the surface of CNCs allow for chemical modification that extend their applications in new fields. These attractive features have enabled CNCs to be used as efficient adsorbents for water treatment [19], reinforcing nanofillers in polymers nanocomposites [20], templates for fabricating chiral nematic mesoporous films [21], supporting materials for loading noble metallic nanoparticles [22], building blocks for layer-by-layer assembly [23], drug delivery vectors [24], as well as bioimaging probes [25]. Particularly, surface modifications of CNCs to impart reversible pH-responsive behaviour and/or fluorescent emission character have received some attention. While pH-responsive CNCs demonstrated facile control over flocculation [26] and hydrogel formation [27], several fluorescently labelled CNCs showed promise in the fields of optical labelling [28] and bioimaging [25,29]. Furthermore, dual fluorescent labelling of CNCs reported by Nielsen and co-workers showed a pH dependent fluorescent response as well as their potential use as pH nanosensors [30].

In this study, intrinsic fluorescent G6 PAMAM dendrimers were grafted onto the surface of CNCs via amide bonds. The final product referred to as CNC-PAMAM, not only demonstrated pH-responsive properties and fluorescent emission, but also retained the encapsulation capabilities of the PAMAM dendrimers. In addition, the pH-responsive property of CNC-PAMAM induced by the pH dependent protonation/deprotonation and stretch–compress properties of PAMAM displayed interesting characteristics that may assist in the regeneration process in some practical applications. Moreover, an investigation on the surface charge of CNC-PAMAM was conducted by examining the interactions between CNC-PAMAM and surfactants (sodium dodecyl sulphate and hexadecyltrimethylammonium bromide) at different pHs. The results obtained from the interactions provided important information pertinent to the application of CNC-PAMAM in the pharmaceutical industry, as cosmetic additives and catalyst carriers. This pH-responsive property, encapsulation capability and fluorescent emission of CNC-PAMAM would find promising applications in the fields of pH-responsive separation, optical imaging, antimicrobial, nanotemplates and biomedicine.

2. Experimental section

2.1. Materials

Generation 6.0 poly (amidoamine) dendrimers (5 wt.% solution in methanol) (G6-PAMAM), N-hydroxysuccinimide (NHS), sodium bromide, sodium dodecyl sulphate (SDS), MES hydrate, Sodium hypochlorite (NaClO, available chlorine, 10–15%), TEMPO, N-(3-dimethylaminopropyl)-N′-ethyl-carbodiimide hydrochloride (EDC) and hexadecyltrimethylammonium bromide (CTAB) were purchased from Sigma–Aldrich and used without further purification. CNCs were supplied by CelluForce Inc., and CNCs are produced based on the protocol reported in the patent [31]. Deionized water from a Milli-Q Millipore system (>18 MΩ cm) was used in the sample preparation.

2.2. Methods

The simple synthetic process of CNC-PAMAM is shown in Scheme 1. It includes the oxidation of CNCs and the grafting of PAMAM dendrimers onto CNCs.

2.3. TEMPO mediated oxidation of cellulose nanocrystals

Grafting of PAMAM via an amide bond requires the primary hydroxyl groups of CNCs to be converted into carboxyl groups. CNCs (5 g) were added to 375 mL of water with TEMPO (0.0125 g) and NaBr (0.125 g), and sonicated for 15 min using a Branson 1510 sonicator to form a stable, homogeneous suspension. NaClO (50 mL) was slowly added to the CNC suspension, and the pH value was maintained at 10.5 throughout the reaction. After stirring for 4 h, ethanol (5 mL) was added to quench the oxidation. The suspension containing oxidized CNCs (CNC—COOH) was dialyzed in deionized water for 72 h to remove excess reactants, and then freeze-dried.

2.4. Grafting reaction of G6-PAMAM onto CNC—COOH

The grafting reaction of G6-PAMAM onto the surface of CNC—COOH was carried out using the carbodiimide-mediated amidation method reported by Sehgal and Vijay [32]. Freeze-dried CNC—COOH (50.0 mg) was homogeneously dispersed in 50 mL of the MES buffer (25 mM, pH 6.5) via sonication for 10 min. Afterwards, EDC (100 mg) and NHS (100 mg) were added slowly to the mixture under sonication. The mixture was reacted under stirring for 2 h and was then added drop-wise to a 10 mL diluted G6 PAMAM dendrimers aqueous solution (containing 500 μL 5 wt.% G6 PAMAM in methanol) under sonicating condition. The final mixture was allowed to react for 24 h. The product was purified by dialysis and ultrafiltration.

2.5. Characterization

Fourier-transform infrared (FT-IR) spectroscopy was employed to study the chemical composition of freeze-dried CNCs, CNC—COOH and CNC-PAMAM. The samples were pressed into KBr pellets and subsequently analysed using a Bruker Tensor 27 spectrometer with a resolution of 4 cm^{-1}. Elemental analysis was conducted using an elemental analyser (Vario Micro Cube CHNS Analyzer, Elementar Americas, Inc.). The morphologies of these samples were examined using a Philip CM 10 transmission electron microscope (TEM) under an accelerating voltage of 60 kV. Approximately 10 μL of a 0.01 wt% sample were sprayed onto carbon-coated copper grids, dried and placed in the vacuum chamber of the TEM. The interaction between CNC-PAMAM and SDS/CTAB was studied using a Microcal VP-isothermal titration calorimeter (Northampton, MA, USA). Control experiments were carried out by titrating surfactants into water under same conditions. Typically, ~282 μL of titrant (SDS or CTAB) was injected at different volume increments into a 1.455 ml CNC-PAMAM (0.04 wt%) solution or water. Zeta potential values were measured using a Malvern Zetasizer. The concentrations and pH values of the samples were controlled and adjusted using a Metrohm 809 Titrando autotitrator, where titrants (0.1 M HCl or 0.1 M NaOH) were introduced at a flow rate of 0.1 mL/min. All samples were sonicated for 5 min and kept for 30 min prior to testing. Electrophoretic mobility μ was measured at 25 °C,